Seabirds as bioindicators of Southern Ocean ecosystems : concentrations of inorganic and organic contaminants, ecological explanation and critical evaluation

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"There are two spiritual dangers in not owning a farm. One is the danger of supposing that breakfast comes from the grocery, and the other that heat comes from the furnace."

Aldo Leopold, *A Sand County Almanac* (1949)

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Preface

Pawel Kuczynski, 2010

List of publications

Papers included in the doctoral dissertation

Paper 1 **Carravieri A.**, Bustamante P., Churlaud C., Fromant A., Cherel Y. Moulting patterns drive within-individual variations of stable isotopes and mercury in seabird body feathers: implications for monitoring of the marine environment. *Marine Biology*. 2014, 161, 963-968.

Paper 2 **Carravieri A.**, Bustamante P., Churlaud C., Cherel Y. Penguins as bioindicators of mercury contamination in the Southern Ocean: birds from the Kerguelen Islands as a case study. *Science of the Total Environment*. 2013, 454-455, 141-148.

Paper 3 **Carravieri A.**, Cherel Y., Blévin P., Brault-Favrou M., Chastel O., Bustamante P. Mercury contamination in a large subantarctic avian community. *Environmental Pollution*, 2014, 190, 51-57.

Paper 4 **Carravieri A.**, Bustamante P., Tartu S., Meillère A., Labadie P., Budzinski H., Peluhet L., Barbraud C., Weimerskirch H., Chastel O., Cherel Y. Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators. *Environmental Science and Technology*, 2014, 48 (24), 14746-14755.

Paper 5 Blévin P., **Carravieri A.**, Jaeger A., Chastel O., Bustamante P., Cherel Y. Wide range of mercury contamination in chicks of Southern Ocean seabirds. *Plos One*, 2013, 8, (1), e54508.

Paper 6 Fromant A., **Carravieri A.**, Bustamante P., Labadie P, Budzinski H., Peluhet L., Churlaud C., Chastel O., Cherel Y. Persistent organic pollutants and trace elements in the tissues of Antarctic prions (*Pachyptila desolata*) at Kerguelen archipelago, southern Indian Ocean. *In preparation for Science of the Total Environment*.

Other papers related to the doctoral work

1) Goutte A., Barbraud C., P., Meillère A., **Carravieri A.**, Bustamante P., Labadie P., Budzinski H., Delord K., Cherel Y., Weimerskirch H., Chastel O. Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. *Proceedings of the Royal Society B*, 2014, 281, 20133313.

2) Costantini D., Meillère A., **Carravieri A.**, Lecomte V., Sorci G., Faivre B., Weimerskirch H., Bustamante P., Labadie P., Budzinski H., Chastel O. Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. *Oecologia*, 2014, 175, 1–10.

Other papers not related to the doctoral work

1) Goutte A., Charassin J-B., Cherel Y., **Carravieri A.**, De Grissac S., Massé G. Importance of ice algal production for top predators: new insights using sea ice biomarkers. *Marine Ecology Progress Series*, 2014, 513, 269–275.

2) Fontaine M., **Carravieri A.**, Simon-Bouhet B., Bustamante P., Gasco N., Frederic Bailleul F., Guinet C., Cherel Y. Ecological tracers and at-sea observations document the foraging ecology of southern long-finned pilot whales (*Globicephala melas edwardii*) in Kerguelen waters. *Marine Biology*, 2015, 162 (1), 207-219.

Contribution to international conferences and workshops

1) **Carravieri A.**, Cherel Y., Blévin P., Bustamante P. Mercury contamination in a large community of subantarctic seabirds: age class and trophic variation amongst species from the Kerguelen Islands. Oral communication, 9th Ecology and Behaviour Meeting, 22-26 April 2013, Strasbourg, France.

2) **Carravieri A.**, Cherel Y., Churlaud C., Bustamante P. Penguins as bioindicators of mercury contamination: geographic and historical trends within the southern Indian Ocean. Oral communication, 8th International Penguin Conference, 2-6 September 2013, Bristol, UK.

3) **Carravieri A.**, Cherel Y., Blévin P., Bustamante P. Mercury contamination in a large community of subantarctic seabirds: age class and trophic variation amongst species from the Kerguelen Islands. Poster, Fall Scholl ORQUE-SUDOE, 8-11 October 2013, Pau, France.

4) **Carravieri A.**, Bustamante P., Tartu S., Meillère A., Labadie P., Budzinski H., Weimerskirch H., Chastel O., Cherel Y. Foraging ecology drives contamination by persistent organic pollutants and mercury in the wandering albatross. Oral communication, 12th Seabird Group Conference, 21-23 March 2014, Oxford, UK. Runner-up prize for best talk.

5) **Carravieri A.**, Bustamante P., Tartu S., Meillère A., Labadie P., Budzinski H., Weimerskirch H., Chastel O., Cherel Y. Persistent organic pollutants and trace elements in blood of the wandering albatross: influence of individual traits and foraging ecology. Oral communication, SETAC Europe 24th Annual Meeting, 11-15 May 2014, Basel, Switzerland.

Abbreviations

Elements

As Arsenic

Ca Calcium

Cd Cadmium

Fe Iron

Hg Mercury

Me-Hg Methyl-mercury

Mn Manganese

Na Sodium

Pb Lead

Se Selenium

Sn Tin

Zn Zinc

Organic contaminants

DDD DichloroDiphenylDichloroethane

DDE DichloroDiphenyldichloroEthylene

DDT DichloroDiphenylTrichloroethane

HCB HexaChloroBenzene

HCH HexaChlorocycloHexane

OCPs Organochlorine Pesticides

PBDEs Polybrominated Diphenyl Ethers

PCBs Polychlorinated Biphenyls

Other abbreviations

Abb. Abbreviation

ACC Antarctic Circumpolar Current

AMAP Arctic Monitoring and Assessment Program

ANR National Agency for Research

CEBC Centre d'Etudes Biologiques de Chizé

dw Dry weight

GC-ECD Gas Chromatography

GC-NCI-MS Gas Chromatography coupled to Mass Spectrometry operated in Negative Chemical Ionisation

GLM Generalized Linear Model

GLS Global Location Sensing

GPS Global Positioning System

HSD Honest Significant Difference

LIENSs Littoral, Environnements et Sociétés

LM Linear Model

LoD Limit of Detection

LoQ Limit of Quantification

MDE Mercury Depletion Event

$\mathbf{R^2_{adj}}$ Adjusted R Squared

SD Standard Deviation

SE Standard Error

-SH Thiol

TAAF Terres Australes et Antarctiques Françaises

TEF Toxic Equivalency Factor

UNEP United Nation Environment Program

vs. *Versus*

WP Work Package

ww Wet weight

Thesis outline

The Polartop project (2011-2015, coordinator Olivier Chastel) is a multidisciplinary research program lead by the Centre d'Etudes Biologiques de Chizé (CEBC) and supported by the French National Agency for Research (ANR). Polartop brings ecotoxicologists, ornithologists, chemists and physiologists together in order to assess the pattern and potential effects of environmental contaminants in avian predators of the French Southern and Antarctic Lands (Terres Australes et Antarctiques Françaises, hereafter **TAAF**). The project develops in five working packages (WP) aiming to describe (WP1, coordinator Hélène Budzinski, LPTC, and WP2, coordinator Paco Bustamante, LIENSs) and explain (WP3, coordinator Yves Cherel, CEBC, **doctoral work of Alice Carravieri** and post-doctoral work of Caio Cipro) environmental contaminant concentrations and patterns and to investigate their effects on the endocrine system (in particular with regards to the stress response, WP4, coordinator Olivier Chastel, doctoral work of Sabrina Tartu) and demography and fitness (WP5, coordinator Christophe Barbraud, post-doctoral work of Aurélie Goutte). **The aim of my doctoral thesis is thus to develop WP3 in close association with both WP1 and WP2.** During the doctoral work I had the chance to go to the Kerguelen Islands and participate to the large Polartop sampling program (see Table A1 in the Appendix). During the fieldwork season, I also participated to other ongoing studies on Kerguelen birds and mammals.

This dissertation has a synthetic approach. It integrates results on different inorganic and organic contaminants and different bird species in order to highlight the main scientific outcomes of my work. The papers issued from the doctoral work, both published and in preparation, together with the Materials and Methods section, are presented in appendices. Given the high number of targeted environmental contaminants (more than 30), the level of in-depth investigation is variable depending on chemicals. For example, non-essential trace elements (*e.g.*, Hg, Cd, Pb) have received more attention than essential ones, because of their

potential toxicity (Polartop has an effect-oriented approach). A large part of this doctoral work has focussed particularly on Hg, because of its peculiar characteristics as a global pervasive and highly-toxic metal that biomagnifies in marine food webs. Moreover, due to an easy and relatively fast analysis technique of total Hg, a large set of analysed samples of penguin and other seabird feathers was already available before the beginning of the Polartop program and, hence, of the thesis. Finally, the doctoral dissertation deliberately focuses on the marine environment, rather than on the terrestrial habitats of the different TAAF districts. Indeed, the avifauna of the TAAF encompasses almost exclusively marine birds. By studying seabirds, contaminant evaluation largely concerns the marine environment and marine food webs, while influences of land are supposed to be minimal.

Specifications on terminology

Ecotoxicology is a fairly new discipline (Truhaut 1977) presenting some terms that are used inconsistently in the literature. For example, the terms "**trace elements**" may have different meanings depending on the scientific discipline or the environmental compartment of interest. Here, "trace elements" will be used to designate both essential and non-essential metals and metalloids, excluding the major (carbon, hydrogen, oxygen and nitrogen) and mineral elements (calcium, Ca; chlorine; magnesium; sodium, Na; phosphorus; and sulphur) that are found in **animal organisms**. The terms "contaminant" and "pollutant" are also loosely used in the literature. In this thesis, the term "**contaminant**" indicates a chemical present at concentrations above those that might be considered "normal" and cause no measurable environmental or biological effect. On the other hand, the term "**pollutant**" designates a chemical that produces damage at the measured concentration. Therefore, a given compound can (or not) be regarded as a contaminant or a pollutant depending on the environmental concentration and context. Some naturally occurring chemicals, such as trace elements, can

thus be considered as contaminants, because human activities have significantly disturbed their natural fluxes, increasing the quantities that are circulating in the environment (Sen and Peucker-Ehrenbrink 2012). In some instances, trace elements can also act as pollutants, especially in the case of massive, localised discharges. Man-made compounds such as organic pesticides and industrial chemicals are usually regarded as pollutants, but can be considered as contaminants when present at very low concentrations in the environment. Here, particular caution will be employed in defining chemicals as contaminants or pollutants, due to the remoteness of the region of study. Nevertheless, "**environmental contaminants**" will designate the set of **both trace elements and man-made chemicals posing potential threats to ecosystems worldwide**. Finally, the terms "bioindicator" and "sentinel" are sometimes used as synonyms in the literature. This is not the case of this dissertation, where "**sentinel**" indicates a particular kind of bioindicator species that shows a strong, negative response to environmental contaminants even at low concentrations, serving as early-warning organism of environmental threat. Other kinds of bioindicators can be identified, depending on their response to contaminants (positive, negative or no effect, Ramade 2007). Given the difficulty in characterising the effects, in the present thesis the term "**bioindicator**" will be used *lato sensu*, **independently on the response of the species**.

Chapter 1

Introduction

Somewhere in the southern Indian Ocean
Photo Thibaut Lacombe

1.1. Environmental contaminants: origin and fate in marine ecosystems

At a global scale, growing anthropogenic activities associated with increasing human population, economic growth and technological development have led to profound modifications of the environment. Together with depletion of natural resources, biodiversity loss and climate change, contaminants are recognized among the main environmental threats of our time (Ramade 2007). Public awareness of the hazards of environmental contamination mainly arose from tragic accidents such as the Minamata mercury (Hg) poisoning in Japan in the 1950s (Grandjean et al. 2010) or the pervasive dioxin contamination started by the explosion of an industrial plant in Seveso, Italy, in 1976 (White and Birnbaum 2009). Increasingly, government regulators and the general public are concerned about the health of the environment, and require indicators that assess its status and trends (Burger and Gochfeld 2004). In order to preserve environmental and human health and assure sustainability of our technological society, the scientific knowledge on the fate and effects of environmental contaminants on individuals and ecosystems is crucial (Ramade 2007).

1.1.1. Definitions and toxic effects in vertebrates

Trace elements. Trace elements encompass mainly metals and metalloids (Table 1). Metals are commonly defined as elements with a lustrous appearance, which are good conductors of electricity, and generally enter chemical reactions as cations (Walker et al. 2012). Metalloids have characteristics of both metals and non-metals. Among trace elements, the **essential** ones, such as iron (Fe), manganese (Mn) or selenium (Se), are involved in physiological and biochemical processes, and are thus subject to homeostatic control. Essential elements are necessary to organisms within a precise, sometimes very narrow range of concentrations (Walker et al. 2012). Outside this "window of essentiality", detrimental effects may emerge, either in terms of nutritional deficiencies or toxicity. On the other hand, **non-essential**

elements, such as cadmium (Cd), Hg or lead (Pb), have no known physiological function and can be toxic at very low concentrations. Notably, the essentiality of trace elements may vary depending on the biological kingdom (*e.g.*, between animals, plants and bacteria) (Walker et al. 2012).

Table 1. *Metal character and essentiality of trace elements in animal organisms and plants (modified from Senesi et al. 1999). The 14 trace elements of interest of this thesis are in* ***bold****.*

Element		Atomic number	Atomic mass	Metal character	Essentiality
Ag	**Silver**	**47**	**108**	**Metal**	**No**
As	**Arsenic**	**33**	**75**	**Metalloid**	**Yes (not plants)**
B	Boron	5	11	Metalloid	Yes (not animals)
Ba	Barium	56	137	Metal	No
Be	Beryllium	4	9	Metal	No
Bi	Bismuth	83	209	Metal	No
Cd	**Cadmium**	**48**	**112**	**Metal**	**No***
Co	**Cobalt**	**27**	**59**	**Metal**	**Yes**
Cr	**Chromium**	**24**	**52**	**Metal**	**Yes (not plants)**
Cu	**Copper**	**29**	**64**	**Metal**	**Yes**
F	Fluorine	9	19	Nonmetal	Yes (not plants)
Fe	**Iron**	**26**	**56**	**Metal**	**Yes**
Hg	**Mercury**	**80**	**201**	**Metal**	**No**
Mn	**Manganese**	**25**	**55**	**Metal**	**Yes**
Mo	Molybdenum	42	96	Metal	Yes
Ni	**Nickel**	**28**	**59**	**Metal**	**Yes (not plants)**
Pb	**Lead**	**82**	**207**	**Metal**	**No**
Sb	Antimony	51	122	Metalloid	No
Se	**Selenium**	**34**	**79**	**Metalloid**	**Yes**
Sn	Tin	50	119	Metal	Yes (not plants)
Ti	Titanium	22	48	Metal	No
Tl	Thallium	81	204	Metal	No
V	**Vanadium**	**23**	**51**	**Metal**	**Yes**
W	Tungsten	74	184	Metal	No
Zn	**Zinc**	**30**	**65**	**Metal**	**Yes**

* Cd has however been discovered to have a biological function in a diatom (*Thalassiosira weissflogii*), where it activates a carbonic anhydrase enzyme (Lane et al. 2005)

At the molecular level, the different affinity of trace elements to major elements, such as oxygen, nitrogen and sulphur, helps in interpreting the biochemical basis of their toxicity

(Nieboer and Richardson 1980). Some non-essential elements (notably Cd and Hg) have the capacity to bind to non-metallic components of cellular macromolecules, such as the sulfhydryl (thiol, -SH) groups of proteins containing the essential amino acid cysteine. Often, toxic effects result from, or are enhanced by, interactions between trace elements. For example, Ca deficiency may lead to increased intestinal absorption of Pb (Goyer 1995), and Cd may induce changes in Zn homeostasis, resulting in increased Zn retention in the liver and/or kidneys (Brzóska and Moniuszko-Jakoniukalker 2001). The toxicity of trace elements depends not only on their concentrations in the organism, but also on their chemical forms (Yokel et al. 2006). For instance, metalloids and some metals (*e.g.*, Hg, Pb, Sn) can bind covalently to organic groups, forming compounds of different degrees of lipophilicity. These organometallic compounds behave differently than the elemental form, and some can be highly toxic (*e.g.*, methyl-mercury, Me-Hg, and organo-tins, organo-Sn, such as tributyltin, TBT, that was used in anti-fouling paint). At the systemic level, different organs and functions are affected by trace elements toxicity. In man and other vertebrates, the kidney is the critical target of Cd toxicity, with tubular cell damage resulting in increased protein excretion in urines (proteinuria) (UNEP 2010a). On the other hand, the central nervous system is primarily affected by both Hg and Pb, resulting in sensory and motor deficits and behavioural impairment (Wolfe et al. 1998, UNEP 2010b). Furthermore, trace elements can exert subtle and/or chronic effects, with likely implications in carcinogenesis, immunotoxicity and endocrine disruption (Silvera et al. 2007, Schroeters et al. 2006, UNEP 2010a,b).

Persistent organic pollutants. Organic (carbon-based) pollutants designate a large group of environmental contaminants of global concern. An international environmental treaty, the **Stockholm Convention**, has established a list of the most harmful organic pollutants, namely "Persistent Organic Pollutants" or POPs. The convention, which was

adopted in 2001 (amendments in 2009 and 2011), establishes an obligation for the 179 ratifying countries to eliminate and restrict the production and use of POPs. These compounds notably comprise the so-called **legacy-POPs**, such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), and **emerging-POPs**, such as polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (Table 2).

Table 2. *POPs included in the Stockholm Convention and their (past) uses. Source: Ritter et al. 1995, Jia et al. 2009 (for endosulfan) and the website of the Stockholm Convention (www.pops.int). The compounds of interest of this thesis are in* ***bold***

Name	Abb.	Measures[1]	Category[1]	(Past) uses
Aldrin		Elimination	Pesticide	Applied to soils to kill termites, grasshoppers, and other insect pests; aldrin is readily and rapidly converted to dieldrin in the environment.
Chlordane		Elimination	Pesticide	A broad-spectrum insecticide also used extensively against termites. Technical chlordane is a mixture of **chlordane**, heptachlor, **nonachlor** and related compounds.
Chlordecone		Elimination	Pesticide	Mainly used as an agriculture insecticide, chemically related to mirex.
Dichlorodiphenyl-trichloroethane	**DDT**	Restriction	Pesticide	Widely used during World War II to protect soldiers and civilians from diseases spread by insects (mainly typhus). Agricultural applications included mainly cotton. Still applied against mosquitoes in several countries to control malaria. **Metabolites** of biological and ecological relevance are **2,4'- and 4,4'-DDT**; 2,4'- and **4,4'-DDD**; and **2,4'- and 4,4'-DDE**.
Dieldrin		Elimination	Pesticide	Used principally to control termites and textile pests, but also against insect-borne diseases and insects living in agricultural soils. Dieldrine derives also from aldrin transformation.
Endosulfan (and related isomers)		Elimination	Pesticide	Insecticide and acaricide used on a variety of food and non-food crops. It is the most recently added POPs to the Stockholm Convention and is still widely used in China and India and other developing countries.
Endrin		Elimination	Pesticide	Applied on leaves of crops such as cotton and grains. Also used to control rodents.

Name	Abb.	Measures[1]	Category[1]	(Past) uses
Heptachlor		Elimination	Pesticide	Primarily used to kill soil insects and termites, but also crop pests and malaria-carrying mosquitoes.
Hexabromobiphenyl		Elimination	Industrial chemical	Mainly used as flame retardant, especially in the 1970s.
Hexa- and heptabromo diphenyl ether	**Hexa- and heptaBDE**	Elimination	Industrial chemical	Mainly used as flame retardant. They may be converted to lower, and possibly more toxic, PBDE congeners by debromination. This thesis has targeted different **PBDEs: BDE-153, BDE-154** (HexaBDE), **BDE-183** (HeptaBDE).
Hexachlorobenzene	HCB	Elimination, Un. release	Pesticide, Industrial chemical, Un. release	Primarily used in agriculture as a fungicide for the protection of wheat and other cereals. Also a by-product of the manufacture of certain industrial chemicals and exists as an impurity in several pesticide formulations.
α-, β-, **γ-hexachlorocyclo hexane**	α-, β-, γ-HCH	Elimination/ Restriction	Pesticide	γ-HCH (lindane) was primarily a broad-spectrum insecticide for soil and wood treatments. It is still used in human pharmaceuticals for control of head lice and scabies. α- and β-HCH were used as pesticides, but are also by-products of lindane.
Mirex		Elimination	Pesticide	Primarily used as an insecticide against ants and termites. Also used as a fire retardant in plastics, rubber, and electrical goods.
Pentachlorobenzene	PeCB	Elimination, Un. release	Pesticide, Industrial chemical, Un. release	Used in many applications: as a fungicide, a flame retardant and as a chemical intermediate. Also produced unintentionally during combustion and industrial processes. Also present as impurities in solvents and pesticides.

Name	Abb.	Measures[1]	Category[1]	(Past) uses
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride	PFOS, PFOS-F	Restriction	Industrial chemical	Widespread applications: electric and electronic parts, firefighting foam, photo imaging, hydraulic fluids and textiles. PFOS is also an unintended degradation product of related anthropogenic chemicals, and still produced in several countries. These compounds have a hydrophilic character, in contrast to most other POPs.
Polychlorinated biphenyls	**PCBs**	Elimination, Un. release	Industrial chemical, Un. release	There are 209 different congeners. PCBs were used in industry as heat exchange fluids, in electric transformers and capacitors, and as additives in paint, carbonless copy paper, and plastics. The present thesis focuses on **indicator PCBs: CB-28, -52, -101, -118, -138, -153 and -180**.
Polychlorinated dibenzo-p-dioxins	PCDDs	Un. release	Un. release	There are 75 different congeners. PCDDs are produced unintentionally due to incomplete combustion (wood, fossil fuel, waste, etc.), and during the manufacture of pesticides and other chlorinated substances.
Polychlorinated dibenzofurans	PCDFs	Un. release	Un. release	There are 135 different congeners. PCDFs are produced unintentionally form many of the same processes that produce PCDDs, to which they are structurally similar.
Tetra- and pentabromo-diphenyl ether	**tetra- and pentaBDE**	Elimination	Industrial chemical	Used as additive flame retardants. The thesis has targeted different **PBDEs: BDE-47, -49, -66, -71** (tetraBDE), **BDE-99, -100** (pentaBDE) and also ***BDE-17, 28,*** *(triBDE) and* ***BDE-209*** *(decaBDE), which are not included in the Stockholm Convention*.
Toxaphene		Elimination	Pesticide	Insecticide used on cotton, cereal grains, fruits, nuts, and vegetables. Also used to control ticks and mites in livestock.

[1] Un. release: unintentional release.

These chemicals share some structural features, such as high proportions of halogen (especially **chlorine** or **bromine**) substitutions on cyclic aliphatic or aromatic rings. They also have physicochemical properties in common, such as structural stability, relatively low vapour pressure and high hydrophobicity. As a consequence, POPs: 1) are **persistent** in the environment, resisting physicochemical and biological degradation; 2) are **highly mobile** in different environmental media, in particular the atmosphere (see next section); and 3) **bioaccumulate** in living organisms and **biomagnify** up food webs (see section 1.1.3.). Importantly, POPs: 4) are **toxic** for human and wildlife, with main deleterious effects to the endocrine and nervous systems, resulting in reproductive impairment and neurodevelopmental and behavioural anomalies (Colborn et al. 1993, Jones and De Voogt 1999, Lundqvist et al. 2006). Furthermore, POPs can cause immunosuppression, increasing susceptibility to disease (De Swart et al. 1996, Grasman 2002). More recently, POPs have also been implicated in metabolic disorders such as diabetes and obesity (Snedeker and Hay 2012) and some compounds are known or suspected carcinogens (Eskenazi et al. 2009). The biochemical basis of POPs toxicity is highly dependent upon their molecular structure and is often associated with a strong affinity for the aryl hydrocarbon receptor, a ligand-dependent intracellular protein that can stimulate gene transcription (Walker et al. 2012). Moreover, POPs toxicity can originate from other biochemical mechanisms, such as Ca homeostasis and Ca-mediated signalling (Tilson et al. 1998, Snedeker and Hay 2012).

1.1.2. Sources, large-scale movements and role of the World Ocean

Trace elements. Trace elements are naturally-occurring chemicals that are normally found in the Earth crust, atmosphere and water masses. Element mass **mobilisation** (the passage from a passive to a potentially active state, Klee and Graedel 2004) at the Earth's surface happens through natural and anthropogenic processes. Natural flows include mainly soil erosion,

volcanic emissions, riverine and eolian transport, sea-salt-spray, and net terrestrial and marine primary productivity. On the other hand, human flows encompass primarily mining and fossil fuel (coal and petroleum) combustion, but also biomass burning and construction of infrastructures (roads, buildings, tunnels) (Ramade 2007, Sen and Peucker-Ehrenbrink 2012).

Increasing evidence from comprehensive biogeochemical studies shows that the contribution of human activities to global fluxes largely exceeds natural processes for several trace elements, including the potentially harmful Hg, Pb and arsenic (As) (Klee and Graedel 2004, Rauch and Pacyna 2009, Sen and Peucker-Ehrenbrink 2012).

The ocean plays a critical role in the biogeochemical cycle of trace elements, which in turn have a predominant place in regulating marine life and the cycling of major elements, as carbon or nitrogen (Coale et al. 1996, Morel and Price 2003, SCOR 2007). The oceanic distribution of trace elements derives from exchanges with the atmosphere and the ocean margins. In the water column, trace elements undergo chemical and biological reactions, and are ultimately removed by burial in marine sediments (Fig. 1).

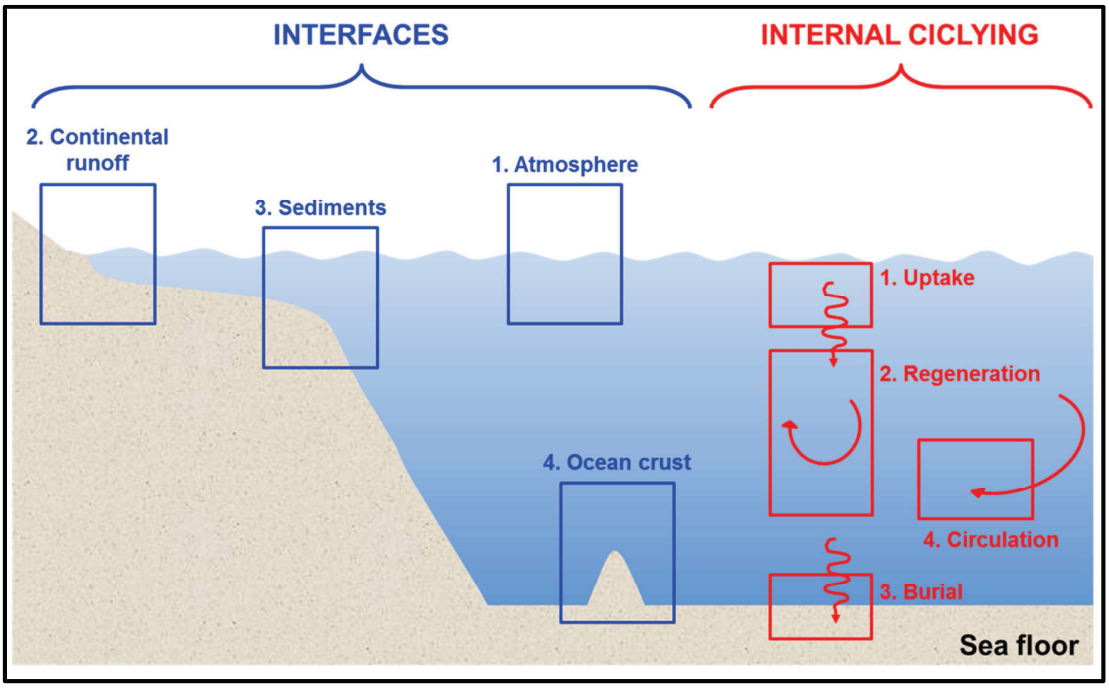

Fig. 1. *Four major ocean interfaces (blue) and four major internal processes (red) are responsible for ocean trace elements distribution (modified from SCOR 2007).*

Long-range transport to the open ocean happens mainly through oceanic currents and atmospheric circulation, followed by wet and dry deposition processes (SCOR 2007). The extent to which trace elements are transported by the atmosphere depends markedly upon their chemical form, which drives their volatility and/or association with atmospheric aerosol particles (Walker et al. 2012). For example, Pb and Cd are mainly associated to aerosol particles and have short atmospheric residence times (days or weeks). However, under particular climatic conditions, they can be transported over intercontinental distances (UNEP 2010a,b, Boyle et al. 2014). Conversely, Hg, under its gaseous **elemental form** (Hg^0) has a very long atmospheric lifetime (0.5-1 year), travelling over a hemispheric to global scale (*e.g.*, Selin 2009). In the open ocean the main source of Hg is atmospheric deposition of the **inorganic form** (Hg^{II}) by wet and dry processes (*e.g.*, Fitzgerald et al. 2007). In seawater, inorganic Hg partitions between the dissolved phase and particulate organic and inorganic matter, and may suffer several abiotic and biotic reactions, which contribute to its cycling in the water column (Fig. 1). These processes may result in the formation of other Hg species, including the return to the elemental form, with re-emission to the atmosphere (~80% of the atmospheric deposited inorganic Hg is re-emitted, Driscoll et al. 2013) and the formation of methylated species, including Me-Hg (mono-methyl Hg), which is easily assimilated by biota (*i.e.* bioavailable, Morel et al. 1998, see section 1.1.3.). The methylation of Hg is mainly driven by **anaerobic microorganisms**, for instance sulphate-reducing and Fe-reducing bacteria (*e.g.*, Hsu-Kim et al. 2013). Thus, Me-Hg production is optimal in suboxic zones, such as the water-sediment interface or low-oxygen regions of the water column. Namely, Me-Hg concentrations generally peak in the mesopelagic zone (200-1000 m depth) (*e.g.*, Fitzgerald et al. 2007).

Persistent organic pollutants. In contrast to trace elements, POPs are mainly of an anthropogenic origin. The major pathways of introduction of POPs in the environment are 1) the intentional application of pesticides over crops and indoor; 2) the production and use in the chemical industry; and 3) the formation of by-products during combustion, which also happens through natural processes (Jones and De Voogt 1999, Walker et al. 2012). Although the first bans of legacy-POPs were made in industrialized countries since the 1970s, **primary and secondary sources of POPs are still present in the environment**. Not only is the production and utilisation of certain POPs exceptionally accepted by the Stockholm Convention, notably for disease vector control in developing countries (*e.g.*, DDT), but releases also occur from stockpiles of obsolete pesticides and industrial chemicals (UNEP 2008).

Two key factors that have made POPs a worldwide problem are their mobility and resistance to photolytic, chemical and biological degradation (Jones and De Voogt 1999). Many POPs are semi-volatile compounds with propensity to enter the gas phase under environmental temperatures. Hence, in warm regions, they may volatilise from soils, vegetation and water bodies into the atmosphere, where they partition between the vapour and particle phases. Lower temperatures favour greater association of POPs to air particles, increasing the likelihood of their deposition on surfaces by wet and dry processes (Ritter et al. 1995). Thus, by repeated air-surface exchange and atmospheric transport, POPs can travel long distances, with deposition being more important at higher latitudes. This phenomenon, known as **global distillation** or "grasshopper effect", results in the accumulation of some POPs in polar regions (Fig. 2, Wania and Mackay 1993, 1996). Moreover, polar environmental conditions favour the persistence of POPs, because the low temperature and winter darkness limit photolytic and biological degradation and ice can entrap them for long periods (Bargagli 2008, Corsolini 2009).

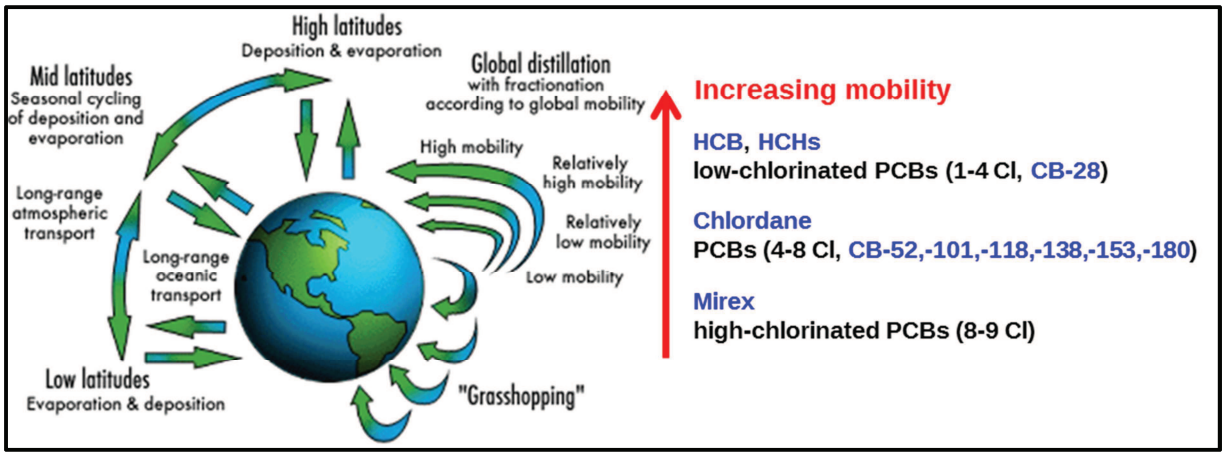

Fig. 2. *Schematic representation of POPs migration processes (modified from Wania and Mackay 1996). Some of the pesticides and PCBs studied during the doctoral work are presented in blue, sorted in increasing mobility.*

Beyond global distillation, other mechanisms are of importance in POPs transport and widespread distribution. Like for trace elements**, oceanic biogeochemical processes have a key role in controlling POPs global dynamics** (Dachs et al. 2002, Nizzetto et al. 2010). In the water column, phytoplankton uptake and adsorption to particulate organic matter constitute a **biological pump** that removes POPs from air-water exchange (Galbán-Malagón et al. 2012), thus influencing atmospheric residence time and grasshopping over the ocean. Consequently, a fraction of POPs may sink to deep waters and sediments, especially in high productivity areas, such as upwelling and high latitude oceanic regions, or becomes incorporated into marine food webs (Jurado and Dachs 2008).

1.1.3. Fate in organisms and food webs

Two concepts are essential to the understanding of the incorporation and fate of environmental contaminants in living organisms: exposure and bioavailability. **Exposure to a contaminant happens when an organism comes into contact with the contaminant**. There are different routes of exposure, depending on the organism and its habitat. In vertebrates, exposure occurs through food and water ingestion, inhalation and dermal contact. **Bioavailability of a contaminant is the fraction that can be *assimilated* following the**

exposure and consequently transferred, stored and/or metabolised by the organism (Ramade 2007). The bioavailability of a contaminant is highly dependent on its chemical form, and is influenced by environmental factors (chemical composition of the medium, association with organic matter, etc.) and intrinsic traits of the organism (nutritional status, mobility, etc.). In vertebrates, contaminants are incorporated through the digestive epithelia, the respiratory surfaces and through the skin (Walker et al. 2012). Importantly, the fraction of the contaminant that transits through the gastrointestinal tract without assimilation is not considered bioavailable. In vertebrates, absorbed contaminants may travel through the bloodstream and be distributed to different tissues where diverse biochemical processes take place. For instance, enzymatic metabolism or **biotransformation**, which occurs mainly in the liver, is an important factor determining the fate of OCPs and PCBs *via* biochemical processes that render them less (**detoxification**) or more (**bioactivation**) toxic and may result in the **excretion** of the formed metabolite(s) (Letcher et al. 2010). On the other hand, trace elements are not biodegradable (except the organic forms to a certain extent), and detoxification in organisms consists of hiding them within proteins such as **metallothioneins**, or depositing them in insoluble forms in intracellular granules, that are excreted or stored over the short to long term (Walker et al. 2012). **Storage** also happens for POPs: given their lipophilicity, reserves of organic compounds can be built in lipid tissues, and consequently released during lipid mobilisation (Borgå et al. 2004). **Bioaccumulation** of contaminants occurs when incorporation overcomes excretion mechanisms, resulting in increasing contaminant concentrations in the organism. Bioaccumulation can happen at all levels of the food web, from primary producers to consumers and decomposers. Furthermore, **biomagnification** occurs when the dietary transfer of a contaminant results in higher concentrations in the consumer than in the food source (Gray 2002). As a consequence, cumulative quantities of contaminants are transferred at each step of the food web, with apex predators exhibiting the

highest concentrations (Fig. 3, Ramade 2007). This phenomenon is often exacerbated in aquatic environments, as a result of two important ecological characteristics (Ramade 2007). **First**, aquatic food webs are generally longer and/or more complex than terrestrial ones, which increases the number of contaminant trophic transfers. **Second**, organisms at the base of aquatic food webs, *i.e.* phyto- and zooplankton, have a high ability to concentrate contaminants from the water. The result is that **contaminants are highly mobile and easily transferred to upper levels of aquatic food webs**. This is particularly worrying for oceanic ecosystems, because, as seen above, the ocean is usually the last sink for several environmental contaminants.

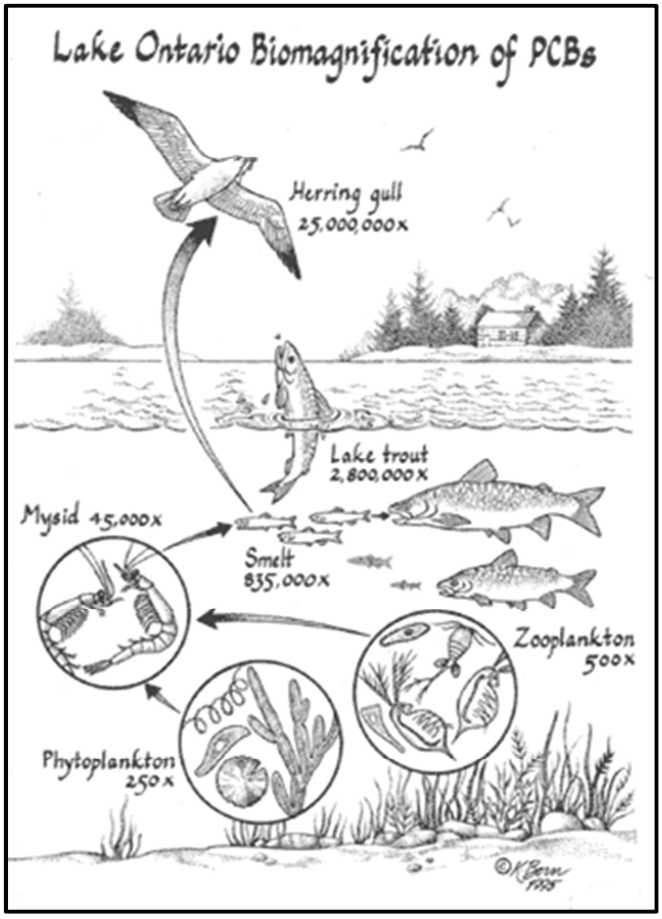

Fig. 3. *A historic example of PCBs biomagnification: the case of Lake Ontario, North America, heavily polluted by PCBs in the 1950s-1970s, where concentrations in herring gulls could be 25 million times higher than those in abiotic matrices (figure from "Our Stolen Future", p. 27, Colborn et al. 1996).*

1.1.4. Birds as bioindicators: a critical role

The "surveillance" or **monitoring** of trace elements and POPs in the environment has become increasingly important in the last decades, given their widespread distribution and potential deleterious effects on human and wildlife. Diverse reasons may solicit environmental monitoring of contaminants, including global or local temporal changes, biological effects on wild populations and hazards to man (Furness 1993). For these purposes, the measure of abiotic matrices, such as water, air and soils, may be expensive and logistically challenging, but above all may give poor insights into the impacts on biota and ecosystems (Burger 1993).

Thus, the monitoring of environmental contamination is often performed through accurately selected living organisms, or **bioindicators**, and the term "biomonitoring" can be applied.

There are three main advantages of using living organisms rather than (or in addition to) abiotic matrices for contaminant monitoring (Ramade 2007). **First**, bioindicators give insights into contaminants bioavailability, which is biologically and ecologically more relevant than inert environmental concentrations. **Second**, several contaminants are found at higher concentrations in organisms than in biotopes, as a consequence of bioaccumulation and biomagnification mechanisms, favouring analytical precision and reliability. **Third**, bioindicators incorporate contaminants over different spatial and temporal scales, giving integrative insights into environmental contamination of food webs. The criteria of choice of bioindicators may change according to the aim of the monitoring or the particular environmental context and will be discussed in Chapter 5.

Birds have historically played a critical role as early-warning organisms (sentinels) of environmental contamination, contributing markedly to the decision to impose international bans and regulations (Walker 2003). For example, the threats of many POPs firstly emerged from reports on adverse effects in wild bird populations, such as organochlorine-induced eggshell thinning and/or birth defects in raptors (especially peregrine falcons *Falco*

Double-crested cormorant with deformed bill from a colony on Lake Michigan, USA (photo from Walker et al. 2012).

peregrinus and bald eagles *Haliaeetus leucocephalus*) and fish-eating birds (notably herring gulls *Larus argentatus* and double-crested cormorants *Phalacrocorax auritus*) in North Europe and North America in the 1960s-1970s (Ratcliffe 1970, Fry 1995, Walker et al. 2012). These facts were notably highlighted in the famous books "*Silent Spring*" by Rachel Carson (1962) or "*Our stolen future*" by Theo Colborn et al. (1996), which greatly contributed to the rise of public awareness of environmental contamination in the USA. Subsequently, birds have been largely used as bioindicators of environmental contamination in a variety of environments (Burger 1993, Furness 1993). This is particularly true for **seabirds**, for several ecological and practical reasons (Furness and Camphuysen 1997, Burger and Gochfeld 2004). First of all, seabirds have **contrasting feeding strategies**, preying at different levels of food webs, which make them ideal models for the assessment of contaminant biomagnification. Many seabirds are **long-lived**, which implies a long period of time for the bioaccumulation of contaminants in internal tissues. Seabirds are usually **colonial and philopatric**, giving the opportunity to follow reproduction easily, to sample several individuals simultaneously and to monitor them repeatedly through time. Seabird species are **widespread**, and can be found even in far-removed, harsh environments. Moreover, seabirds are often **wide-ranging**, thus integrate environmental contaminants over large scales, reaching remote regions that would be very difficult to monitor otherwise. While some of these characteristics may represent drawbacks for environmental monitoring (for example the wide-ranging nature of many seabirds prevents the identification of point sources of exposure), the accurate choice of bioindicator species

and in-depth knowledge on their physiology and ecology may help in interpreting the measured concentrations (Furness 1993, Burger and Gochfeld 2004).

1.2. The Southern Ocean and its avian predators

1.2.1. Oceanographic features

The Southern Ocean comprises the southernmost waters of the World Ocean, surrounding unbroken the Antarctic continent. The precise northern limits of the Southern Ocean have been debated for long (Laws 1985, Knox 1994). This is linked to the fact that the Southern Ocean is not delimited by continents to the north, but by a hydrological structure: the **Antarctic Circumpolar Current (ACC)**. The ACC circulates eastward under the action of westerly winds (found approximately between 45°-55°S) and Earth rotation (Coriolis Force), and has a critical role in the global oceanic circulation (Orsi et al. 1995). Waters found at the edge of the northern limit of the ACC show important gradients in temperature and salinity, constituting a physical delimitation between the Southern and the Atlantic, Indian and Pacific Oceans (Orsi et al. 1995).

Under the influence of the ACC, surface waters of the Southern Ocean have an annular structure, made of three main circumpolar water masses: the **Subtropical**, **Subantarctic** and **Antarctic Zones**. Each one of these zones has relatively uniform, specific physicochemical properties (temperature, salinity and pressure).

The sharp changes in water-mass properties at the edge of the different zones are marked by large scale oceanographic structures: the **fronts**. The **Subtropical Front** delimits the Subtropical and the Subantarctic Zones, while the **Polar Front** divides the Subantarctic and Antarctic Zones (Fig. 4) (Park and Gambéroni 1997, Pollard et al. 2002). In this doctoral thesis, **the Southern Ocean is considered to comprise all the water masses situated**

between the Subtropical Front and the Antarctic continent. The remote islands and archipelagos of the TAAF are situated in the Indian sector of the Southern Ocean, from Terre Adélie in Antarctica, to the Kerguelen and Crozet archipelagos in the Subantarctic Zone, the first in the immediate vicinity of the Polar Front and the second further north, up to Amsterdam Island in the Subtropical Zone. Since Amsterdam Island is located north of the Subtropical Front, it is outside the Southern Ocean *stricto sensu*, but for simplicity it will be considered as a Southern Ocean locality in this doctoral dissertation. TAAF localities are therefore distributed along a large latitudinal gradient with oceanographic characteristics that are similar to those of the other sectors of the Southern Ocean.

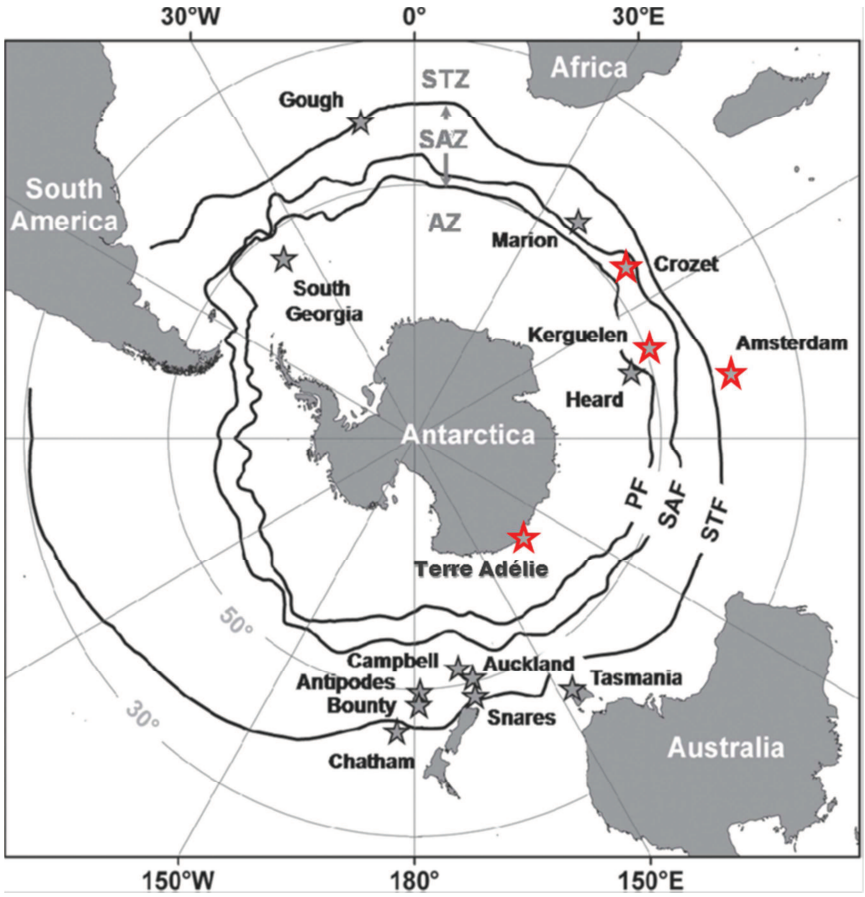

Fig. 4. *Map of Southern Ocean waters with the main islands and archipelagos and oceanographic features (fronts: PF, Polar Front; SAF, Subantarctic Front; STF, Subtropical Front; zones: AZ, Antarctic Zone; SAZ, Subantarctic Zone; STZ, Subtropical Zone) (Modified from Cherel et al. 2013).*

Another important feature of the Southern Ocean is related to its biochemistry. The Southern Ocean is indeed the largest high-nutrient low-chlorophyll region of the World Ocean

(Boyd et al. 2000, Blain et al. 2001, Sanial et al. 2014). While nutrient concentrations (nitrate, phosphate, and silicic acid) are high, phytoplankton development is generally low in these waters. This paradoxical pattern is mainly linked to low water concentrations of Fe (Boyd et al. 2000), but other limiting factors, such as light penetration, temperature and zooplankton grazing are involved (Boyd 2002). Some areas of the Southern Ocean are however extremely productive. For instance, large phytoplankton blooms are seasonally observed eastward of the Crozet, Kerguelen and South Georgia Islands (Blain et al. 2001, Korb and Whitehouse 2004, Sanial et al. 2014), as the result of an "island fertilisation" effect. Namely, Fe and other micro- and macronutrients are released by sediments deposited on the margins of the islands and transported by ACC advection, creating phytoplankton plumes of usually high mesoscale (spatial scale of ~10 km) and temporal variability. High biological productivity results also from releases of benthic Fe and other nutrients from shallow bathymetry areas, such as the **Kerguelen Plateau** (Blain et al. 2007, Mongin et al. 2009). Marine areas at the retreating pack-ice edge are also characterized by phytoplankton blooms during the austral summer (Bargagli 2008). Southern Ocean waters are thus characterised by an important spatio-temporal variability in biological production, but contribute significantly to the global primary productivity budget. Accordingly, Southern Ocean phytoplankton blooms constitute an important sink for atmospheric carbon dioxide, playing a critical role in the regulation of the global climate (Blain et al. 2007). The biological pump is also crucial for environmental contaminants' scavenging and sedimentation, as shown in Arctic and Antarctic waters (Galbán-Malagón et al. 2012, 2013a,b), even if this has not yet been addressed in large regions of the Southern Ocean.

1.2.2. Southern Ocean seabirds: cycle and feeding habits

Due to its abundant stocks of potential prey, the Southern Ocean hosts a tremendous biomass and diversity of wildlife, including huge populations of avian predators. More than 300 million seabirds breed within this ocean (Van Franeker et al. 1997), of which several millions in the TAAF (*e.g.*, Weimerskirch et al. 1989), including rare and endangered species, such as the Amsterdam albatross *Diomedea amsterdamensis* and the Indian yellow-nosed albatross *Thalassarche carteri* (IUCN Red List 2011). Different orders of birds have their breeding grounds in the TAAF: the Sphenisciformes (penguins), the Procellariiformes (albatrosses and petrels), the Charadriiformes (skuas, gulls and terns) and the Pelecaniformes (cormorants), with also one representative of the Anseriformes (ducks). The Sphenisciformes and the Procellariiformes dominate the avian community in terms of biomass and species diversity, respectively (Warham 1990, Williams 1995), and include iconic species such as the emperor penguin *Aptenodytes forsteri* and the wandering albatross *Diomedea exulans*.

The annual life cycle of these seabirds is characterised by three energetically-demanding processes: reproduction, migration and moult. Usually, these processes are temporally separated, in order to optimise energy expenditure (King 1974).

Reproduction occurs during the productive austral summer for most species, while only a few are winter breeders (*e.g.*, emperor penguins and grey petrels *Procellaria cinerea*, Table 3), or have long breeding periods of approximately one year (*e.g.*, king penguins *Aptenodytes patagonicus* and wandering albatrosses). TAAF seabirds have a low reproductive outcome, raising generally one chick per year (Warham 1990, Williams 1995). **Migration** strategies are highly variable, with some subantarctic species being resident on their breeding islands (*e.g.*, gentoo penguins *Pygoscelis papua*, Kerguelen cormorants *Phalacrocorax verrucosus* and terrestrial species, Table 3), while others migrate over different distances (from Southern Ocean to subtropical, tropical and trans-equatorial destinations, up to the

Northern Atlantic and Pacific Oceans) between breeding events (*e.g.*, Marchant and Higgins 1990). **Moult** is also a critical phase of seabirds' life cycle. Regular feather replacement is essential to the maintenance of thermic insulation and flight performance (*e.g.*, Bridge 2006). Pattern, duration and timing of moult differ widely amongst TAAF seabird species. Notably, flying seabirds replace their feathers **sequentially** over a protracted period (two-three months to one year, in general, Bridge 2006) during the inter-breeding season. On the other hand, penguins, which are flightless, diving seabirds, have a unique moulting pattern among birds: they renew all their feathers **simultaneously**, just before or just after the breeding period (Williams 1995). Importantly, penguins fast during moult, since transient reduction in thermal insulation prevents them from going at sea (Groscolas and Cherel 1992, Cherel et al. 1994a).

Since prey availability is spatially and temporally variable in Southern Ocean waters, seabirds have evolved a wide range of contrasting feeding strategies, in order to exploit their heterogeneous environment while limiting between-species competition. Penguins and diving petrels have evolved exceptional diving capacities, exploiting the water column from **pelagic** to **benthic** environments, depending on species (Table 3). On the other hand, flying seabirds explore the marine environment horizontally, feeding from **neritic** (coastal and peri-insular) to **oceanic** waters (Table 3). Some species of oceanic seabirds can travel thousands of kilometres over short periods (days), encompassing large latitudinal gradients (*e.g.*, Weimerskirch et al. 2014).

During reproduction, Southern Ocean seabirds are **central-place foragers** (Orians and Pearson 1979): they alternate periods of feeding at sea with periods at the breeding site for pairing, incubation, and the care and feeding of their offspring.

Thus, seabird diet has been mainly evaluated through **chick food** during the breeding period, because parent birds carry significant quantities of prey items in their stomach at that time only (*e.g.*, Cherel et al. 2000a). Overall the main prey are few key species of marine

organisms, including mainly swarming crustaceans (euphausiids, hyperiid amphipods), schooling and neritic fish (myctophids and notothenioids, respectively) and cephalopods (oceanic squids), with some seabirds, such as skuas (*Chataracta* genus) and giant petrels (*Macronectes* genus), relying extensively on carrion (Ridoux 1994, Guinet et al. 1996, Cherel and Hobson 2005, Cherel et al. 2010 and other references in Table 3). Outside the breeding period, seabirds are no more constraint to return to the nest and can disperse far from breeding grounds, with feeding strategies being largely unknown at that time. While in some instances migrating grounds have been identified thanks to satellite tracking (*e.g.*, Bost et al. 2009, Thiebot et al. 2012), the stable isotopes technique (*e.g.*, Cherel et al. 2000b, Cherel et al. 2006, see Chapter 2) and/or direct observations (*e.g.*, Weimerskirch et al. 1985), the diet composition and feeding habitats of most species outside the breeding period remain largely unknown.

King penguin colony on the Crozet Islands (photo Alice Carravieri)

Table 3. *Feeding habits and morphological characteristics of the 34 species investigated in the doctoral dissertation.*

Species	Abb.	District [a]	Size (cm)[b]	Mass (kg)[b]	Foraging habitat			Chick food	References
					Breeding (horizontal; vertical)	Non-breeding	Moulting (adult feather isotopes)		
Spheniscidae									
Emperor penguin (*Aptenodytes forsteri*)	EP	TA	120	30.5	Oceanic; pelagic, benthic	Unknown (likely oceanic)	Antarctic	Fish	Offredo and Ridoux (1986); Cherel (2008)
King penguin (*Aptenodytes patagonicus*)	KP	KER, CRO	90	13.3	Oceanic; epi-mesopelagic	Unknown (likely oceanic)	Polar Front	Mesopelagic fish	Bost el al. (2002); Cherel et al. (2010)
Gentoo penguin (*Pygoscelis papua*)	GP	KER, CRO	83	6.5	Neritic (open sea); pelagic, benthic	Resident all year	Neritic waters	Benthic fish (pelagic crustaceans)	Lescroël and Bost (2005)
Adélie penguin (*Pygoscelis adeliae*)	AP	TA	70	6	Neritic; epipelagic	Antarctic Zone, oceanic	Antarctic	Crustacean (fish)	Wienecke et al. (2000); Cherel (2008)
Macaroni penguin (*Eudyptes chrysolophus*)	MP	KER, CRO	71	4.9	Neritic, oceanic; epipelagic	Polar Frontal Zone, oceanic	Subantarctic	Pelagic crustaceans (fish)	Cherel et al. (2010), Bost et al. (2009); Thiebot et al. (2011a,b)
Southern rockhopper penguin (*Eudyptes chrysocome filholi*)	SRP	KER, CRO	50	2.9	Neritic (closed sea); epipelagic	Subantarctic and Polar Frontal Zone, oceanic	Subantarctic	Pelagic crustaceans (fish)	Tremblay and Cherel (2000, 2003); Cherel et al. (2010); Thiebot et al. (2012)
Northern rockhopper penguin (*Eudyptes chrysocome moseleyi*)	NRP	AMS	53	2.9	Neritic; epipelagic	Subtropical Zone	Subtropics	Squid and crustaceans	Tremblay and Cherel (2003) ; Thiebot et al. (2012)
Diomedeidae									
Wandering albatross (*Diomedea exulans*)	WA	KER, CRO	123	8.8	Oceanic; sea surface	Subtropical Zone, oceanic	Subtropics	Fish and cephalopods	Cherel and Weimerskirch (1999); Cherel et al. (2013)
Amsterdam albatross (*Diomedea amsterdamensis*)	AA	AMS	110	6.5	Oceanic; sea surface	Subtropical Zone, oceanic	Subtropics	Fish and cephalopods	Cherel et al. (2013)
Black-browed albatross (*Thalassarche melanophrys*)	BBA	KER	88	3.8	Neritic (open sea); sea surface	Subtropical Zone (southern Australia), neritic	Subtropics	Benthopelagic fish (cephalopods)	Cherel et al. (2000a,b); Cherel et al. (2013)
Indian yellow-nosed albatross (*Thalassarche carteri*)	YNA	AMS	76	2.3	Oceanic; sea surface	Subtropical Zone (southern Australia), neritic	Subtropics	Fish (cephalopods)	Pinaud et al. (2005); Cherel et al. (2013)
Light-mantled sooty albatross (*Phoebetria palpebrata*)	LMSA	KER	84	3.1	Oceanic; sea surface	Subantarctic Zone; oceanic	Subantarctic, Antarctic	Cephalopods (crustaceans, carrion)	Ridoux (1994); Cherel et al. (2013)

Species	Abb.	District [a]	Size (cm)[b]	Mass (kg)[b]	Foraging habitat			Chick food	References
					Breeding (horizontal; vertical)	Non-breeding	Moulting (adult feather isotopes)		
Procellariidae									
Northern giant petrel (*Macronectes halli*)	NGP	KER	88	4.4	On land and at sea surface	Subantarctic to Subtropical Zones, oceanic	Subantarctic	Carrion, seabirds	Ridoux (1994); Thiers et al. (2014)
Grey petrel (*Procellaria cinerea*)	GrP	KER	50	1.1	Oceanic; sea surface	Unknown	Subtropical Front	Fish (cephalopods)	Ridoux (1994)
White-chinned petrel (*Procellaria aequinoctialis*)	WCP	CRO	55	1.3	Oceanic; sea surface	Northern waters (Benguela current), neritic	Subtropics (Benguela)	Fish (cephalopods, crustaceans)	Catard et al. (2000); Delord et al. (2010); Péron et al. (2010)
Great-winged petrel (*Pterodroma macroptera*)	GWP	KER	39	0.56	Oceanic; sea surface	Unknown	Subtropics	Cephalopods (crustaceans)	Ridoux (1994)
White-headed petrel (*Pterodroma lessonii*)	WHP	KER	43	0.70	Oceanic; sea surface	Unknown	Subantarctic	Fish (cephalopods)	Zotier (1990)
Soft-plumaged petrel (*Pterodroma mollis*)	SPP	KER	35	0.30	Oceanic; sea surface	Unknown	Subtropics	Fish (cephalopods, crustaceans)	Unpublished data
Kerguelen petrel (*Aphrodroma brevirostris*)	KeP	KER	35	0.36	Oceanic; sea surface	Unknown	High Antarctic	Crustaceans	Ridoux (1994)
Snow petrel (*Pagodroma nivea*)	SP	TA	35	0.29	Oceanic; sea surface	Unknown	Antarctic	Fish (crustaceans)	Ridoux and Offredo (1989)
Blue petrel (*Halobaena caerulea*)	BP	KER	29	0.20	Oceanic; sea surface	Antarctic Zone, oceanic	High Antarctic	Crustaceans (mesopelagic fish)	Cherel et al. (2002a); Cherel et al. (2006)
Antarctic prion (*Pachyptila desolata*)	AnP	KER	26	0.16	Oceanic; sea surface	Subtropical Zone, oceanic	Subtropics	Crustaceans	Cherel et al. (2002b) ; Cherel et al. (2006)
Thin-billed prion (*Pachyptila belcheri*)	TBP	KER	26	0.15	Oceanic; sea surface	Antarctic Zone, oceanic	High Antarctic	Crustaceans	Cherel et al. (2002b) ; Cherel et al. (2006)
Hydrobatidae									
Wilson's storm petrel (*Oceanites oceanicus*)	WSP	KER	17	0.039	Neritic; sea surface	Unknown	Subtropics	Crustaceans	Ridoux (1994)
Black-bellied storm petrel (*Fregetta tropica*)	BBSP	KER	20	0.053	Neritic, oceanic; sea surface	Unknown	Subtropics	Crustaceans and carrion (squid, fish)	Ridoux (1994)
Grey-backed storm petrel (*Garrodia nereis*)	GBSP	KER	18	0.033	Neritic; sea surface	Unknown	Subtropics	Crustaceans	Ridoux (1994)

Species	Abb.	District [a]	Size (cm)[b]	Mass (kg)[b]	Foraging habitat			Chick food	References
					Breeding (horizontal; vertical)	Non-breeding	Moulting (adult feather isotopes)		
Pelecanoididae									
Common diving petrel (*Pelecanoides urinatrix*)	CDP	KER	23	0.14	Neritic (closed sea); epipelagic	Unknown	Subantarctic, low Antarctic	Crustaceans	Bocher et al. (2000)
South Georgian diving petrel (*Pelecanoides georgicus*)	SGDP	KER	20	0.12	Oceanic; epipelagic	Unknown	Subantarctic, low Antarctic	Crustaceans	Bocher et al. (2000)
Phalacrocoracidae									
Kerguelen shag (*Phalacrocorax verrucosus*)	KS	KER	65	1.9	Neritic (open sea); benthic	Resident all year	Neritic waters	Benthic fish	Watanabe et al. (2011)
Stercorariidae									
Subantarctic skua (*Catharacta lönnbergi*)	SS	KER, CRO, AMS	58	1.9	On land and at sea surface	Unknown	Subtropics	Small petrels	Mougeot et al. (1998)
Antarctic skua (*Catharacta maccormicki)*	AS	TA	53	1.2	On land and at sea surface	Northern Hemisphere	Northern Hemisphere	Seabird eggs and chicks, fish	Unpublished data
Laridae									
Kelp gull (*Larus dominicanus*)	KG	KER	60	1.1	On land and at sea surface	Resident all year	Coastal	Carrion, seabirds (limpets)	Stahl and Mougin (1986)
Chionididae									
Lesser sheathbill (*Chionis minor*)	LS	KER	40	0.83	On land	Resident all year	Terrestrial, coastal	Carrion, eggs, invertebrates, algae	Jouventin et al. (1996)
Anatidae									
Kerguelen Pintail (*Anas eatoni eatoni*)	KPi	KER	40	0.48	On land	Resident all year	Terrestrial	Vegetation	Unpublished data

[a] TAAF district where the species was sampled. Abbreviations: TA, Terre Adélie; KER, Kerguelen Islands; CRO, Crozet Islands; AMS, Amsterdam Island.
[b] Species average for size (length from the tip of the bill to the end of the tail) and mass (body weight) from *Shirihai et al (2002)*.

TAAF birds are thus potentially exceptional candidates as bioindicators of environmental contamination in the Southern Ocean. Indeed, by targeting different age classes and species with diverse breeding locations and foraging ranges, a variety of spatial scales can be investigated, from Antarctica to the subtropics (**latitudinal dimension**), including neritic and oceanic environments (**horizontal dimension**), and benthic and pelagic environments (**vertical dimension**). Furthermore, different seasons can also be targeted by choosing species breeding at different times of the year (for example summer *versus* (*vs.*) winter breeders). Finally, by sampling different tissues, different temporal integrations of environmental contaminants can be assessed, including past exposure over migrating grounds.

1.3. Main objective and outline of the thesis

Due to its pristine reputation, harsh environmental conditions, and remote, immense extension, the Southern Ocean has been poorly characterised in terms of environmental contaminants, in contrast with other marine environments (Galbán-Malagón et al. 2013b). Investigations on contaminant concentrations have been mainly carried out on the Antarctic continent and in its coastal waters (*e.g.*, Bargagli 2008, Corsolini 2009), while the subantarctic and subtropical environments have been scarcely documented. The paucity of data concerns particularly the Indian sector of the Southern Ocean. At the start of this doctoral thesis, trace elements (Bustamante et al. 1998a,b, 2003, Bocher et al. 2003, Scheifler et al. 2005) and POPs (Monod et al. 1992, 1995, Guruge et al. 2001a,b, Tanabe et al. 2004, Tao et al. 2006, Noël et al. 2009) had been measured in only some marine biota, with also limited information on seawater concentrations (Joiris and Overloop 1991, Iwata et al. 1993, Cossa et al. 2011). Environmental contaminants were also determined in freshwater salmonids of the Kerguelen Islands (Jaffal et al. 2011a,b). However, only recent studies used high-resolution

mass spectrometric detectors for POPs compound-specific analyses, with investigations realised in the early 1990s likely presenting methodological pitfalls.

In this context, **the main goal of my doctoral work is to make a general assessment of environmental contamination in the southern Indian Ocean by using birds as bioindicators and to identify the best candidate species for future biomonitoring surveys**.

More precisely, the doctoral work involves 1) a **descriptive step**, aiming to assess and compare environmental contaminant concentrations in TAAF birds to those of other species and regions, and 2) an **explanatory step**, aiming to evaluate the observed concentrations in the light of both intrinsic (individual traits) and extrinsic (feeding strategies) factors. To this end, blood and/or body feathers have been non-destructively collected from a high number of birds breeding in the TAAF (**34 avian species**). **In the present dissertation, I have chosen to focus mainly on the explanatory step**, but descriptive aspects will also be discussed.

Importantly, the state of the art, the questions, hypotheses and predictions of my doctoral work will not be detailed here, but, instead, in each chapter of the dissertation, together with the main results and intermediate conclusions.

The **methodological approach** of the doctoral work will be presented in **Chapter 2**, including results of a method paper (**Paper 1** in the Appendix). The **explanatory step** of the doctoral work will be the object of **Chapter 3**, which encompasses results of different papers (**Paper 2 to 5**). In **Chapter 4**, selected seabird species will be used to depict **spatial and temporal trends** of environmental contamination in the southern Indian Ocean. This fourth part is highly integrative, including results from **Papers 4 and 5** and unpublished work. The main conclusions and perspectives of the doctoral work, including the descriptive aspects, will be presented in **Chapter 5**. This last part will notably set the main scientific outcomes of the doctoral work in the context of the other ongoing investigations of the Polartop project.

Chapter 2

Methodological approach: sampling blood and feathers and the stable isotope method

Moulting northern rockhopper penguin on Amsterdam Island
Photo Jérémie Demay

2.1. Advantages of the non-destructive sampling of blood and feathers

In the past decades, ecotoxicological investigations on birds were conducted mainly on internal organs, because the large mass of tissue available made it possible to identify and quantify several compounds. More recently, however, with the development of highly sensitive analytical techniques, the use of non-destructive methods has greatly increased. In addition to obvious ethical reasons, the non-destructive collection of blood and feathers in birds makes it possible to: **first**, sample a large number of individuals and species over a short period of time; **second**, realise longitudinal studies, by sampling the same individuals over time; and **third**, measure other compounds that could be related to environmental contaminant concentrations, such as trophic tracers (see below).

During the doctoral work, environmental contaminant concentrations and stable isotope ratios of carbon and nitrogen have been measured in **34 avian species** from the TAAF, by using **blood and/or body feathers** from **adult** birds and/or **chicks** (**full-feathered pre-fledging chicks**, *i.e.* just before they become independent), amounting to **1472 individuals** (Table A1 in the Appendix).

2.2. How do environmental contaminants partition between internal organs and blood?

Environmental contaminant exposure in seabirds occurs *via* food intake, while the dermal and respiratory routes are considered to be negligible (Burger and Gochfeld 2004). Similarly, due to generally very low concentrations in water when compared to prey (Ramade 2007), uptake from water is also supposed to be minimal. After ingestion, the dynamics of environmental contaminants in seabirds involve absorption in the intestine, **transport via the bloodstream**, accumulation in internal tissues (*e.g.*, liver, kidney, muscle and fat, Scheuhammer 1987, Wolfe et al. 1998, Borgå et al. 2004) with **redistribution to the plumage** during feather

growth (*e.g.*, Furness et al. 1986), and elimination in eggs (*e.g.*, Agusa et al. 2005), excreta (*e.g.*, Ancora et al. 2002) and preen oil (Yamashita et al. 2007) (Fig. 5).

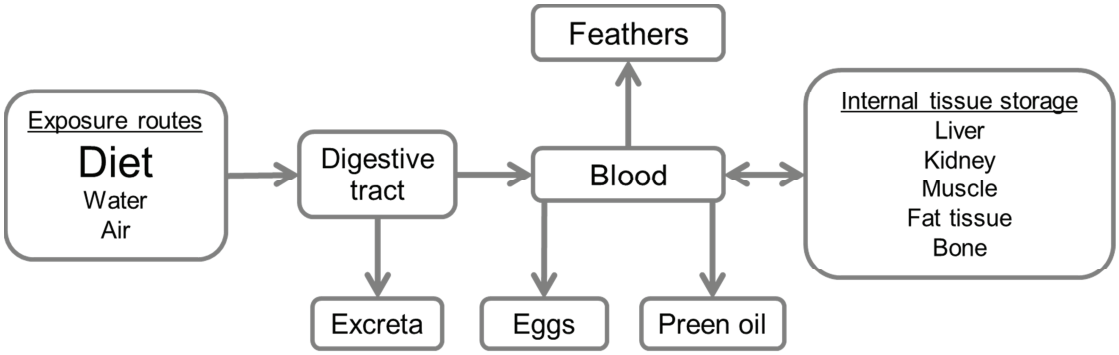

Fig. 5. *Simplified model of environmental contaminants dynamics in seabirds. Modified from Monteiro and Furness (1995).*

Given its role as a transport medium, correlations between environmental contaminant concentrations in internal organs and blood have been frequently observed, especially for Hg and POPs (*e.g.*, Henriksen et al. 1998, Eagles-Smith et al. 2008, Szumiło et al. 2013).

Therefore, in my doctoral work, I have assumed that **blood is a good proxy of environmental contaminant burdens over the short-term (weeks to months)**. This assumption has some limitations that will be thoroughly discussed in Chapter 5.

Importantly, trace elements and POPs were measured in **red blood cells** and **plasma**, respectively, where they preferentially partition (Matthews et al. 1984, Keller et al. 2004, Coeurdassier et al. 2012, Tavares et al. 2013). Hence, within the text, "blood" refers either to red blood cells for trace elements and plasma for POPs, while in figures I have reported the respective blood compartments.

Furthermore, **total Hg** has been measured in blood and feathers. Since **Me-Hg** accounts for more than 95% of blood and feather Hg burdens in birds (*e.g.*, Thompson and Furness 1989, Evers et al. 2008, Bond and Diamond 2009a), **total Hg was considered to approximate Me-Hg**. Thus, in the text "Hg" refers to total Hg, unless otherwise indicated.

2.3. How are environmental contaminants deposited into feathers?

Feathers are unique epidermal structures made of beta-keratin (*e.g.*, Stettenheim 2000). Distal areas emerge first from the feather follicle, whereas proximal aspects form sequentially until the structure is complete (Burger and Gochfeld 1997). During growth (**two-three weeks**, Burger 1993), each feather shaft contains an axial artery connecting the developing cells to the circulatory system. Different bloodborne materials are thus sequestered in the growing feather: structural compounds, such as amino acids (**functional deposition**) and non-structural chemicals (**incidental deposition**), such as hormones and environmental contaminants (Burger 2003, Bortolotti 2010, García-Fernández et al. 2013). The blood supply completely atrophies once the feathers are fully grown, leaving them as inert, long-term archives of the sequestered compounds (Burger and Gochfeld 1997). Some trace elements accumulate particularly in feathers, because of their affinity to –SH groups of keratin. This is the case of Hg, with 50% to 90% of the total body burden being excreted in feathers during moult (*e.g.*, Honda et al. 1986, Braune and Gaskin 1987).

Feather Hg concentrations encompass both dietary intake during feather synthesis and accumulated burdens in internal tissues since the last moult (*e.g.*, Furness et al. 1986). In my doctoral work, I have extensively used feathers for Hg measurement in TAAF seabirds, assuming that **the plumage is a good proxy of Hg burdens accumulated over the inter-moult period**. Conversely, the temporal integration of other trace elements and POPs into feathers are poorly known (Agusa et al. 2005, Jaspers et al. 2007).

2.4. Why using the stable isotope method?

Since environmental contaminants are incorporated almost exclusively from the diet, assessing seabird feeding strategies is critical to understand their contaminant exposure (*e.g.*,

Becker et al. 2002, Anderson et al. 2010, Leat et al. 2013). In order to evaluate the trophic ecology of a large number of species, conventional studies such as satellite tracking and stomach content analysis can be difficult and expensive to realise. Furthermore, they suffer an important bias: they do not directly inform on *assimilated food*. A powerful alternative to conventional techniques is the stable isotope method. The isotopic technique is based on the concept that an animal's chemical composition reflects that of its diet in a predictable manner (Kelly 2000). For example, consumers are enriched in ^{15}N relative to their food and consequently **stable nitrogen signatures ($^{15}N/^{14}N$, $\delta^{15}N$)** serve as indicators of their **trophic position** (Vanderklift and Ponsard 2003). By contrast, **stable carbon signatures ($^{13}C/^{12}C$, $\delta^{13}C$)** vary little within food webs and, in the marine environment, $\delta^{13}C$ values are mainly used to indicate the **foraging habitats** of predators, including seabirds (Cherel and Hobson 2007, Cherel et al. 2013). The isotopic method has already been validated in the southern Indian Ocean, with $\delta^{15}N$ values of seabirds increasing with trophic level (Cherel et al. 2010). Moreover, Southern Ocean **oceanic waters** are marked by a well-defined latitudinal $\delta^{13}C$ gradient of particulate organic matter (Trull and Armand 2001) that is reflected in the tissue of consumers (Cherel and Hobson 2007, Jaeger et al. 2010a, Quillfeldt et al. 2010). By measuring the $\delta^{13}C$ signature of seabirds, it is thus possible to estimate their main foraging zones, depending on the targeted tissues (Fig. 6): based on blood (and feather) $\delta^{13}C$ isoscapes, values less than -22.9 ‰ (-21.2 ‰), -22.9 to -20.1 ‰ (-21.2 to -18.3 ‰), and greater than -20.1 ‰ (-18.3 ‰) are considered to correspond to the Antarctic, Subantarctic and Subtropical Zones, respectively (Jaeger et al. 2010a). Furthermore, in the vicinity of islands, $\delta^{13}C$ signatures can also depict neritic (high $\delta^{13}C$ values) *vs.* oceanic (low $\delta^{13}C$ values) consumers (Cherel and Hobson 2007). Importantly, the stable isotope method enables estimating seabirds feeding strategies over different temporal scales, depending on the sampled tissue and its protein turnover rate (Kelly 2000).

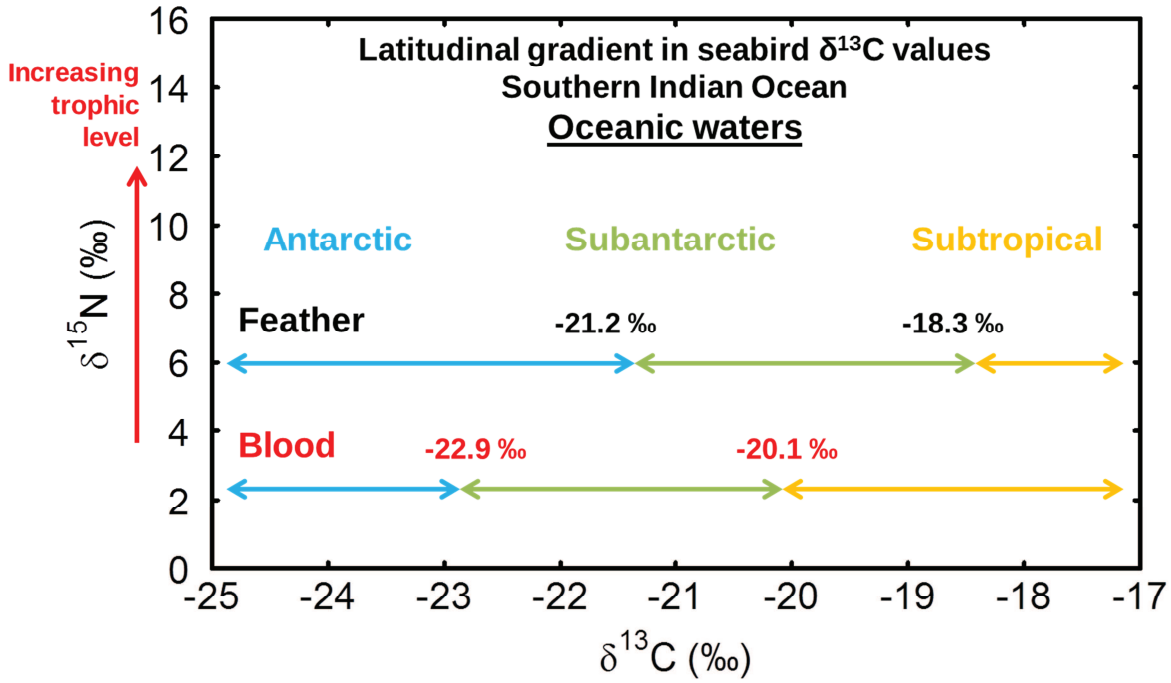

Fig. 6. *Schematic representation of the position of the main **oceanic** water masses of the southern Indian Ocean as depicted by seabird blood and feathers $\delta^{13}C$ values (values from Cherel et al. 2014, based on Jaeger et al. 2010a).*

In this doctoral work, isotopic signatures were determined in **red blood cells**, which reflect dietary intake over approximately **one to two months** preceding sampling, depending on bird size (Hobson and Clark 1992, 1993, Carleton and Del Rio 2005). Isotopic signatures were also measured in **body feathers**, which reflect feeding ecology over (or just preceding, for penguins) the **moulting period**, since feathers are metabolically inert after their synthesis (Cherel et al. 2000b, Bearhop et al. 2002).

2.5. The problem of synchronous *vs.* asynchronous moults

This doctoral work makes use extensively of **body feathers** to measure Hg in seabirds. The **first step** of my doctoral work has thus consisted in a **methodological investigation** on the reliability of body feathers as biomonitoring tissues.

State of the art and hypothesis. Ideally, a monitoring tool must show little within- and between-individual variations in the concentrations of targeted compounds in order to

facilitate the statistical description of spatio-temporal trends within a population. **Body feathers are generally considered as the best feather type to sample**, for ethical reasons, since their removal does not impair flying ability, but also for scientific reasons, since they are more homogeneous and more representatives of the entire plumage than flight or tail feathers (Furness et al. 1986, Burger and Gochfeld 1992, Jaeger et al. 2009). Thus, I have decided to look at the within-individual heterogeneity of stable isotope values and Hg concentrations in body feathers, which has rarely been addressed before (Thompson et al. 1993, Bond and Diamond 2008, Jaeger et al. 2009, 2010b, Brasso et al. 2013). Since environmental contaminants and stable isotopes are deposited in the plumage during feather growth, the timing, duration and pattern of moult are critical in driving contaminant concentrations and isotopic signatures in feathers (*e.g.*, Furness et al. 1986, Thompson et al. 1998a, Kelly 2000). Indeed, changes in foraging habitat or diet during moult lead to large variations in feather $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Jaeger et al. 2010b), and feathers that grow at different times present different Hg concentrations, as the Hg body pool is progressively depleted during the moult (Furness et al. 1986, Braune and Gaskin 1987). As illustrated in the introduction (section 1.2.2.), most TAAF seabird species moult sequentially over protracted periods, whereas **penguins** has a synchronous moult. A simultaneous moult occurs also in **chicks** of all species, which grow new body feathers towards the end of the rearing period. *A synchronous growth theoretically means that all body feathers should have the same chemical composition and thus should show identical stable isotope values and contaminant concentrations, i.e. low within-individual variability.* In order to test this hypothesis, I compared within-individual differences (four feathers per individual) in stable isotope Hg values in three model species having synchronous (king penguin adults and white-chinned petrel *Procellaria aequinoctialis* chicks) and asynchronous (Antarctic prion *Pachyptila desolata* adults) moulting patterns of body feathers (**Paper 1** in the Appendix).

Results and discussion. As expected, the Antarctic prion adults had significantly higher within-individual variation in stable isotopes signatures and Hg concentrations than king penguin adults and white-chinned petrel chicks. This is clearly illustrated by the much larger standard deviations (SD) of body feather $\delta^{13}C$ and Hg values in the different species (Fig. 7). High within-individual variability of stable isotopes and Hg values complicates the use of body feathers as effective monitoring tools by blurring spatio-temporal trends within a population (Bond and Diamond 2008). Within-individual variations in species with asynchronous moult should be quantified and then, according to the scientific goal, multiple body feathers could be pooled in order to perform a unique measurement per bird (Jaeger et al. 2009, Brasso et al. 2013).

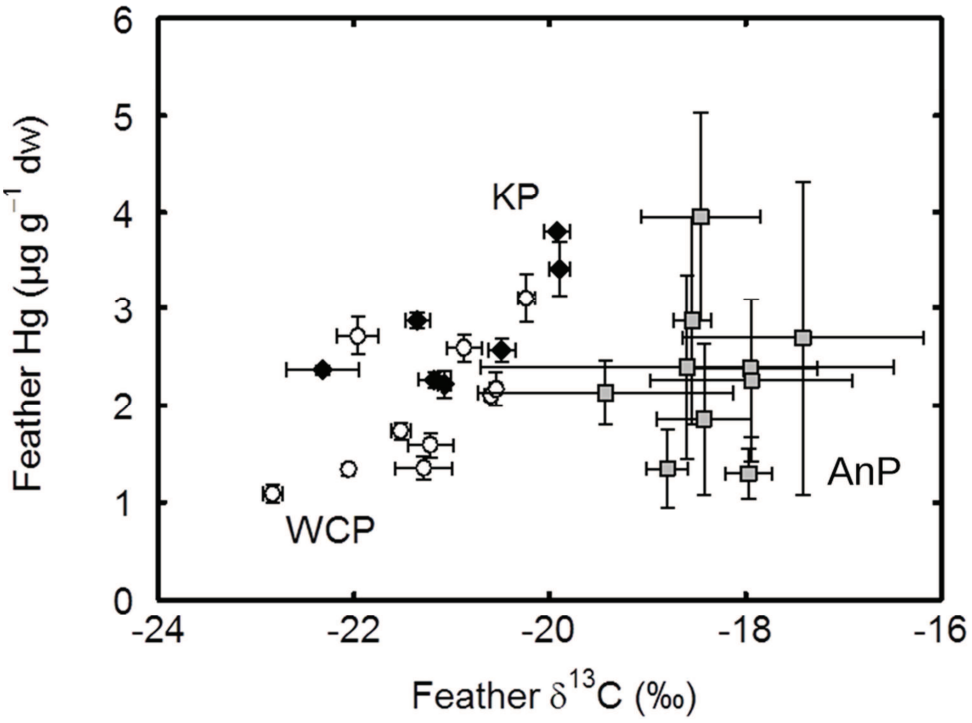

Fig. 7. *Simultaneously moulting white-chinned petrel chicks (WCP; white circles) and king penguin adults (KP; black diamonds) show smaller SD in both feather $\delta^{13}C$ and Hg values than sequentially moulting Antarctic prion adults (AnP; grey squares). Values are mean ± SD of four body feathers per individual bird. Abbreviation: dw, dry weight. Modified from Fig. 1 in **Paper 1**.*

On the other hand, the extremely low variation in stable isotopes and Hg values in species with synchronous moulting patterns has three interesting implications. **First**, **all** body feathers that grow simultaneously have the same $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations.

Second, the analysis of any quantity of pooled body feathers is equally representative of the individual, including **one single body feather**. **Third**, there should be negligible within-individual variations in other useful stable isotope values of keratin, like δ^2H and $\delta^{34}S$ (Hobson 2011, Ramos et al. 2013), and in the concentrations of other environmental contaminants, including both trace elements and POPs (Burger 1993, García-Fernández et al. 2013).

Conclusion. Long-term routine monitoring investigations on the trophic structure and environmental contamination of ecosystems should focus on birds presenting synchronous rather than asynchronous moult of body feathers, because of their low within-individual heterogeneity. This means targeting chicks rather than adults (see below), and, in the Southern Hemisphere, adult penguins rather than adult flying seabirds.

2.6. The problem of adults *vs.* chicks

Ecotoxicological investigations in seabirds are often conducted on adults (*e.g.*, Anderson et al. 2009, 2010). Nevertheless, my doctoral work has focussed extensively on pre-fledging chicks, because, in agreement with Stewart et al. (1997) and Burger and Gochfeld (2004), they present several advantages for environmental contaminant evaluation and monitoring.

- **First**, most seabird chicks can be easily handled, since they remain on land. Chick blood and feathers can be sampled before fledging with limited disturbance (for example during the annual ringing session in a long-term study colony).

- **Second**, the food of chicks and the foraging ecology of parent seabirds during the chick-rearing period are relatively well-known, as illustrated in the introduction (section 1.2.2.). Thus environmental contaminant concentrations in chicks can be more easily related to dietary sources and represent primarily the **local environment**.

- **Third**, chicks have a simultaneous moult (see above), which is easy to study and represents a **well-defined time window**, because growth of body feathers takes place on land in the mid to the second half of the chick-rearing period (Bost and Jouventin 1991, Phillips and Hamer 2000).

- **Fourth**, the **period of exposure** is perfectly known for chicks, since the timing and duration of their rearing periods are well-known.

- **Fifth**, working on chicks minimizes the temporal mismatch resulting from different integration times between stable isotopes and environmental contaminants in feathers (see also Chapter 5 for this issue) (Bearhop et al. 2000a). This is the case of Hg, with concentrations in feathers reflecting accumulated burdens during the inter-moult period and $\delta^{13}C$ and $\delta^{15}N$ values reflecting feeding ecology during feather growth only (*e.g.*, Bond 2010).

Investigating environmental contaminant concentrations in TAAF seabird adults is also useful, especially in order to evaluate the exposure of the species over its annual life-cycle. Among adult birds, **penguins** have been largely used in my doctoral work, because they present undeniable advantages over adults of flying species.

- **First**, unlike most albatrosses and petrels that disperse in northern waters during the nonbreeding period (BirdLife International 2004), TAAF penguins are restricted to the Southern Ocean all year long and show relatively **constant isotopic niches** (Cherel et al. 2007, Ballard et al. 2010, Thiebot et al. 2012, 2011a,b).

- **Second**, depending on species, penguins forage at different depths of the water column, namely in the epi-, meso-pelagic and benthic zones that can present heterogeneous distributions of environmental contaminants, especially for Hg (*e.g.*, Fitzgerald et al. 2007, Cossa et al. 2011).

- **Third**, penguins have a synchronous moult of all feathers (see above), during which they are easily accessible on land.

Hence, penguins appear to be good models to evaluate environmental contaminant concentrations in their foraging environment. However, adults of flying species merit also attention, since they enable evaluating environmental contamination of food webs in far-removed open-ocean water masses. This is the case of adults of the wandering albatross, which have been widely used in my doctoral work for the investigation of explanatory factors of contaminant exposure and bioaccumulation (see next chapter).

2.7. Summary

- **Blood** and **body feathers** are good monitoring tissues of environmental contaminant concentrations in seabirds.
- Birds with a **synchronous moult** of body feathers are potentially good candidates as bioindicator species of environmental contamination.
- **Penguins** and **chicks** of all species have thus been largely used in my doctoral work to assess geographical and temporal trends of environmental contaminants in TAAF marine food webs.

Chapter 3

What are the main explanatory factors of contaminant exposure and bioaccumulation?

Couple of wandering albatrosses on the Crozet Islands
Photo Olivier Lamy

3.1. Intrinsic *vs.* extrinsic factors driving variation in seabird contaminant concentrations

Many different factors may drive variation in contaminant concentrations in seabirds (*e.g.*, Burger 1993, Furness 1993, Borgå et al. 2004), including:

- intrinsic factors, *i.e.* those related to physiology, such as individual traits (sex, age, size), body condition (lipid stores), and mechanisms related to the toxicokinetics and toxicodynamics of environmental contaminants (absorption, biotransformation, excretion);

- extrinsic factors, *i.e.* those related to the environment and the food source, such as trophic position, foraging habitat and external input of environmental contaminants, including atmospheric deposition on the surface of feathers (*e.g.*, Dauwe et al. 2003, Jaspers et al. 2004).

Understanding the sources of variation of environmental contaminant concentrations in seabirds is crucial for using them as bioindicators of environmental contamination. Namely, if intrinsic factors are not controlled, it is impossible to link reliably concentrations found in seabird tissues with environmental processes. Similarly, if extrinsic factors are not understood, spatio-temporal trends could be associated to the wrong geographic or temporal units. During my doctoral work, different factors of variation have been considered, depending on environmental contaminants and species (Table 4). The influence of body condition could not be investigated, because biometric measures were not taken for a large part of the sampled individuals. The influence of external contamination on feathers was not studied, with feathers being vigorously washed in organic solvents, in order to better evaluate intrinsic deposition of environmental contaminants.

Table 4. *Intrinsic and extrinsic factors potentially influencing environmental contaminant concentrations in seabirds. The factors investigated in the different species during the doctoral work are indicated. Abbreviations: TE, trace elements other than Hg.*

	Wandering albatross	Kerguelen community	TAAF penguins (all species)	TAAF petrels (selected species)
Intrinsic factors				
Age class	-	Hg	Hg	-
Adult age	Hg, Cd, POPs	-	-	-
Sex	Hg, Cd, POPs	-	-	Hg, TE, POPs (Antarctic prions)
Reproductive status	Hg, Cd, POPs	-	-	-
Size	-	Hg	-	-
Lipid	POPs	-	-	-
Body condition	-	-	-	-
Extrinsic factors				
Foraging habitat	Hg, Cd, POPs	Hg (chicks)	Hg (adults); Hg, TE, POPs (chicks)	Hg, TE, POPs (chicks)
Trophic position	Hg, Cd, POPs	Hg (chicks)	Hg (adults); Hg, TE, POPs (chicks)	Hg, TE, POPs (chicks)
Feather external contamination	-	-	-	-

As indicated in Table 4, intrinsic and extrinsic factors of environmental contaminant concentrations have been deeply investigated in the **wandering albatross** (see below). Several sections of the present chapter, in particular those focussing on intrinsic factors, will thus report results from the wandering albatross study (**Paper 4**). On the other hand, given the importance of diet in driving environmental contaminant exposure in seabirds, feeding strategies have been extensively studied in almost all TAAF species (**Paper 2 to 5**). Since intrinsic and extrinsic factors operate simultaneously on environmental contaminant exposure and bioaccumulation, it is difficult to investigate any factor independently from the others,

especially in free-living individuals. This was not the object of my doctoral work, which has a correlative and multi-factorial approach. In this chapter, I decided to present the different factors separately for the sake of clarity and in order to illustrate the thought process necessary to understand the more complex, global picture. Multi-factorial interactions are however discussed when necessary. The rationale and hypothesis linked to each factor will be given in the corresponding sections.

3.2. Why using the wandering albatross to assess the influence of intrinsic and extrinsic factors on variation in environmental contaminant concentrations?

The wandering albatross has particular life-history traits, such as a very long life span (> 50 years), late sexual maturity (~seven years), low reproductive output (one chick in alternate years) and slow moulting patterns, with body feathers being replaced over a complete cycle of breeding and inter-nuptial migration (Marchant and Higgins 1990), *i.e.* two years, while flight feathers need two or more cycles to be completely replaced (Weimerskirch 1991), *i.e.* four years or more. The breeding cycle of wandering albatrosses lasts a complete year, with individuals that are successful in fledging a chick breeding in alternate years (they take a sabbatical year, during which they stay at sea). Conversely, individuals failing during incubation or during the early stages of the chick-rearing period breed again in the following year (Tickell 1968). During the austral summer, at colonies one can find both breeding and non-breeding individuals, including immature birds (pre-breeders), birds that failed reproduction at early stages and mature birds that did not engage in reproduction. Furthermore, this species also shows sex- and age-related variations in feeding strategies during the breeding period: males forage in subantarctic and Antarctic waters, adventuring further south as they get older, whereas females rely on northern warmer waters throughout

their lives (*e.g.*, Lecomte et al. 2010, Jaeger et al. 2014). The wandering albatross is thus an interesting model for studying variation in environmental contaminant concentration with age, sex, breeding status and foraging behaviour. Importantly, the CEBC has been leading an exceptional long-term capture-mark-recapture survey on the wandering albatross population of the Crozet Islands since the 1960s (Weimerskirch et al. 1997), with several chicks being banded for identification every year. Therefore, information on individual traits and breeding history are available for a large number of individuals.

3.3. Does age affect environmental contaminant concentrations in blood and feathers?

According to the phenomenon of bioaccumulation, the longer a seabird is exposed and incorporates bioaccumulative environmental contaminants, the higher should be the burdens in its tissues. Thus *seabirds could be expected to bear increasing concentrations of bioaccumulative environmental contaminants as they get older,* in particular for long-lived species. According to differential accumulation of environmental contaminants in internal organs (see Chapter 2), age-related variation may depend on the tissue analysed. In my doctoral work, I have looked at age-related variations in environmental contaminant concentrations in **blood** and **body feathers (hereafter feathers)** in order to test if age-correction should be applied when using these tissues for biomonitoring. In a first step, age-related changes in environmental contaminant concentrations were studied by comparing chicks and adults, which differ markedly in the time interval of exposure. In a second step I have looked more finely at potential variations in aging adults.

3.3.1. Age-class differences in Hg concentrations in feathers

In this doctoral work, the influence of age class on environmental contaminant concentrations has been assessed for Hg in feathers of seabirds from the Kerguelen Islands (**Papers 2 and 3**). This large avian community (Weimerskirch et al. 1989) offers the opportunity to investigate age-class differences in Hg concentrations in a high number of species with different morphological and ecological adaptations.

State of the art and hypothesis. Several studies on seabirds have shown that adults have higher feather Hg concentrations than chicks at fledging (*e.g.*, Thompson et al. 1991, Bargagli et al. 1998, Burger and Gochfeld 2000, Catry et al. 2008, Bond and Diamond 2009b). As illustrated in Chapter 2, Hg concentrations in feathers represent Hg exposure in the period of time elapsed since the last moult. This period of time encompasses, for **adults**, the **inter-moult period**, which generally lasts one year (Warham 1990, Williams 1995) and, for chicks, the **chick-rearing period** (*e.g.*, Burger 1993), which is of variable duration depending on species (~2-9 months in Kerguelen seabirds). Hg can be transferred from the mother to the chick *via* the egg (*e.g.*, Lewis et al. 1993, Agusa et al. 2005). However, this inherited Hg burden is excreted, at least partially, in the chick down (*e.g.*, Bearhop et al. 2000a, Burger and Gochfeld 2000, Becker et al. 1993) and is highly diluted in internal tissues during growth (Ackerman et al. 2011). Since the time interval available for Hg accumulation is longer in adults than in chicks, *feather Hg concentrations were expected to be higher in adults than in chicks in the Kerguelen community*. Accordingly, *species with short (vs. long) chick-rearing periods were expected to show high (vs. low) adult-to-chick ratios in feather Hg concentrations.*

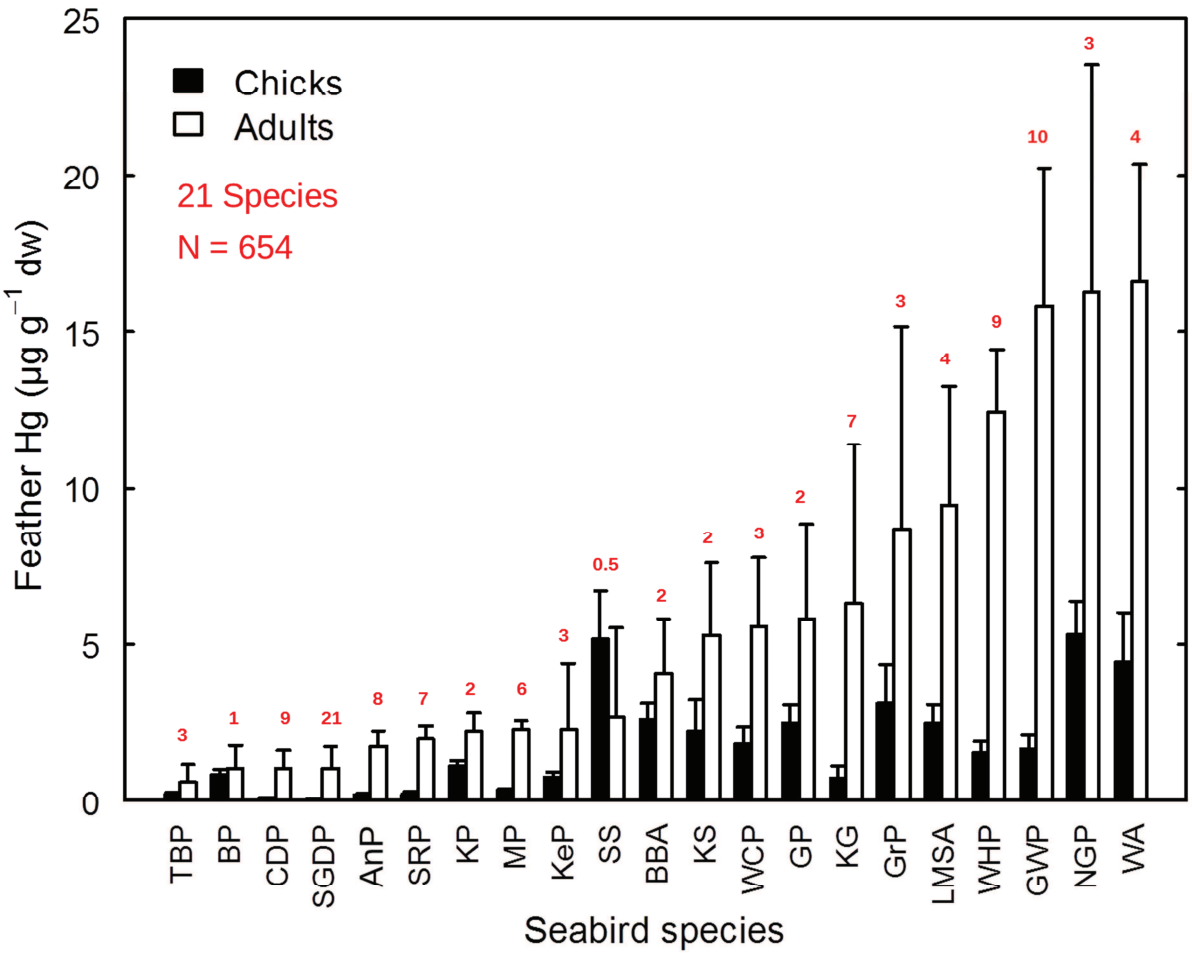

Fig. 8. *Bar plot of adult and chick feather Hg concentrations within the Kerguelen seabird community with adult-to-chick ratios in red. All age-class differences were significant except for blue petrels (Wilcoxon, $p > 0.05$). Note that subantarctic skua adults had higher feather Hg concentrations than chicks (Wilcoxon, $p < 0.05$). Values are means + SD. Abbreviations: TBP, thin-billed prion; BP, blue petrel; CDP, common diving petrel; SGDP, South Georgian diving petrel; AnP, Antarctic prion; SRP, southern rockhopper penguin; KP, king penguin; MP, macaroni penguin; KeP, Kerguelen petrel; SS, subantarctic skua; BBA, black-browed albatross; KS, Kerguelen shag; WCP, white-chinned petrel; GP, gentoo penguin; KG, kelp gull; GrP, grey petrel; LMSA, light-mantled sooty albatross; WHP, white-headed petrel; GWP, great-winged petrel; NGP, northern giant petrel; WA, wandering albatross. Modified from Fig. 3 in **Paper 3**.*

Results and discussion. Feather Hg concentrations were higher in adults than in chicks in almost all Kerguelen species (Fig. 8), which is consistent with a longer duration of exposure and bioaccumulation in adults. Adult-to-chick ratios were highly variable among species (range 0.5-21, Fig. 8), with the hypothesis being verified in most cases. For example, diving petrels, which have short chick-rearing periods (~2 months), showed high ratios (≥ 9), while species such as the wandering albatross, northern giant petrel *Macronectes halli* and king penguin, with very long chick-rearing periods (~9 months), showed low ratios (~2-4).

Nevertheless, there were exceptions to this pattern, as observed in blue petrels *Halobaena caerulea*, subantarctic skuas *Catharacta lönnbergi* and gentoo penguins (ratios: 1.3, 0.5 and 2.4, respectively, despite short chick-rearing periods of ~2-3 months). These exceptions are likely indicative of differential relative Hg exposures in the two age classes. Indeed, seabirds can present parent-offspring dietary segregation, as adults tend to provision their chicks with larger and more energy-rich prey than those they capture for self-feeding (*e.g.*, Alonso et al. 2012). Moreover, adults may rely on different prey during and outside the breeding period. This is likely the case of **subantarctic skuas**, with chicks and adults feeding extensively on bird meat during the chick-rearing period (Mougeot et al. 1998), while moulting adults probably do not rely on other seabirds for feeding, but on low trophic-level prey, as shown in skua populations breeding at other localities (Phillips et al. 2007).

Conclusion. Feather Hg concentrations are higher in adults than in chicks, which indirectly confirms that the time interval of exposure is critical in driving Hg bioaccumulation in internal tissues. However, exceptions exist to this general trend, as shown by the paradoxical results of the subantarctic skua: the **time of exposure and feeding habits** must be taken into account to explain age-class differences in Hg accumulation.

3.3.2. Adult age: case study of the wandering albatross

Limited information is available on the relationship between **adult age** and environmental contaminant concentrations in seabirds because of the difficulty in their age determination, that can be reliably done only by ringing individuals as chicks (Bustnes et al. 2003, Agusa et al. 2005, Mallory et al. 2006). In order to perform a cross-sectional study on the relationship between environmental contaminant concentrations in **blood** and adult age, my doctoral work has made advantage of the long-term data set on the wandering albatross from the Crozet Islands.

State of the art and hypothesis. The investigation of age-related variation in environmental contaminant concentrations in seabirds has produced contrasting results. In most studies on blood and feathers, **no age-related variation** was detected for trace elements (*e.g.*, Furness et al. 1990, Thompson et al. 1991, Becker et al. 2002) and POPs (*e.g.*, Auman et al. 1997, Gonzáles-Solís et al. 2002, Mora et al. 1993) concentrations. In particular, several studies have shown that blood POPs concentrations reach a steady state before the age of breeding (Van den Brink et al. 1998, Bustnes et al. 2003, Ólafsdóttir et al. 2005). Nevertheless, other investigations have also shown **age-related increases** (Hutton 1981, Gochfeld et al. 1996, Donaldson et al. 1997) and **age-related decreases** (Gochfeld et al. 1996, Agusa et al. 2005) of environmental contaminants in seabird internal organs and feathers. Despite these contrasting results, *I expected to find increasing concentrations of Hg and POPs in blood of the wandering albatross* for four main reasons: **first**, this species is exposed to and accumulates high quantities of contaminants, especially Hg (*e.g.*, Hindell et al. 1999, Anderson et al. 2009); **second**, Hg and POPs have well-known bioaccumulative properties in living organisms; **third**, this species has an extremely long life span; and **fourth**, concentrations in blood reflect internal tissue burdens (*e.g.*, Henriksen et al. 1998, Eagles-Smith et al. 2008). *No hypothesis was formulated for the other trace elements, as knowledge on the relationships between internal tissues and feathers concentrations is still limited.* From the very large data set available (N = 180), the age-contaminants relationship was investigated only on the subsample of **breeding individuals** (N = 95 or 75 depending on compounds), in order to compare birds in the same reproductive status.

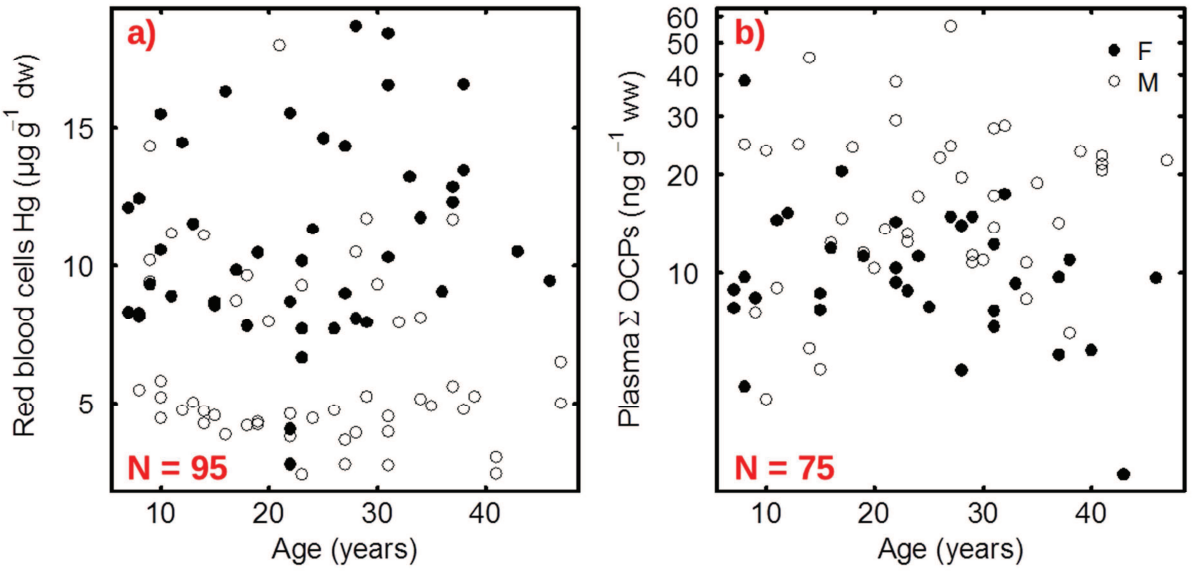

Fig. 9. *Blood **a)** Hg and **b)** Σ_{11}OCPs (on a logarithmic scale) concentrations are not related to adult age in breeding wandering albatrosses from the Crozet Islands. OCPs: cis-chlordane, trans-nonachlor, HCB, γ-HCH, heptachlor, mirex, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT. The effect of age on blood contaminant concentrations was statistically tested through multi-factorial linear models (LMs) (Hg) and generalised linear models (GLMs) (POPs) including other variables, such as sex and feeding habitat (δ^{13}C) (more details in the next sections and in Paper 4). Abbreviations: F, females; M, males. Data from **Paper 4**.*

Results and discussion. Contrary to my prediction, blood concentrations of Hg and the sum of 11 OCPs (Σ_{11}OCPs) were not age-related in breeding wandering albatrosses, despite the presence of very old individuals (> 35 years) (Fig. 9). This was also the case of the sum of 7 PCBs (Σ_7PCBs) and Cd, with age having a weak explanatory power in all model selections (see **Paper 4** for more details). This is in agreement with the idea that seabirds have efficient detoxification mechanisms, including trace elements and POPs feather excretion, POPs preen oil excretion, Me-Hg demethylation and POPs biotransformation (see Chapter 2), even when contaminant exposure is high. This contrasts with results in marine mammals, especially males, which have shown clear age-dependent increases in adult internal tissues contaminant concentrations, including blood (Muir et al. 1999, Sjödin et al. 2000, Lahaye et al. 2006, Correa et al. 2013). Hair, like feathers, is an effective excretory route of environmental contaminants in seal and fur seals (*e.g.*, Dietz et al. 2011), but many marine mammal species, such as baleen and toothed whales, have no fur and thus lack a considerable

opportunity of detoxification and elimination of contaminants. Consequently, they have developed other detoxification mechanisms such as the co-precipitation of Hg with Se (Cuvin-Aralar and Furness 1991).

The contrasting results found for some environmental contaminants and/or species, may derive from the fact that many studies had small sample sizes, limited age ranges and/or neglected potential age-related changes in feeding ecology (*i.e.* exposure). For instance, the age-related decrease of blood Hg concentrations shown in snow petrels *Pagodroma nivea* in the context of the Polartop project was likely linked to age-dependent variations in feeding strategies (Tartu et al. 2014). Feeding habits have been taken into account in the doctoral work on the wandering albatross and are discussed below (section 3.5.2.2.). It must also be noted that the cross-sectional nature of this study does not necessarily reveal information on the contaminant concentrations of individuals as they age (Binnington and Wania 2014). However, similar quantities of blood contaminant residues have been reported in seabirds sampled repeatedly in different years (Auman et al. 1997, Bustnes et al. 2003). Importantly, POPs, Hg and Cd did not affect mortality in the wandering albatross (Goutte et al. 2014a), which excludes potential bias from differential survival of the most contaminated individuals.

Conclusions. Overall, concentrations of Hg and POPs in seabirds increase from the chick stage to the age of maturity and then stay in equilibrium between the organism and exposure routes throughout their lives. The absence of confounding age-related variation in concentrations enhances the value of adult seabirds' blood as a reliable biomonitoring tool of these, and potentially other, environmental contaminants.

3.4. Do other potential physiological differences affect contaminant concentrations in blood and feathers?

3.4.1. Gender

In the present doctoral thesis, sexual differences in environmental contaminant concentrations were tested in the wandering albatross, a species that shows sexual size dimorphism, with male being larger than females (Shaffer et al. 2001), and sex-related variations in feeding strategies (*e.g.*, Jaeger et al. 2014, see section 3.2.).

State of the art and hypothesis. Sexual differences in environmental contaminant concentrations may derive from different non-exclusive factors (Robinson et al. 2012): 1) **egg excretion** of females, in particular for Hg and POPs (*e.g.*, Lewis et al. 1993, Verreault et al. 2006, Bourgeon et al. 2013), a route of elimination males lack; 2) **feeding habits**, which may be driven by sexual size dimorphism, with the larger gender consuming likely larger prey, which could bear higher contaminant burdens (Evers et al. 1998); 3) gender-specific **physiological traits**, such as organ size or metabolic capacities (Robinson et al. 2012). **Wandering albatrosses were sampled during the incubation period**, *i.e.* just after females could excrete part of their environmental contaminant burden into the egg. *Blood concentrations of Hg and POPs were therefore hypothesised to be lower in females than in males*, also considering that females are smaller than males. Conversely, no hypothesis was formulated for the other trace elements, which have egg deposition of variable efficiencies (*e.g.*, Agusa et al. 2005).

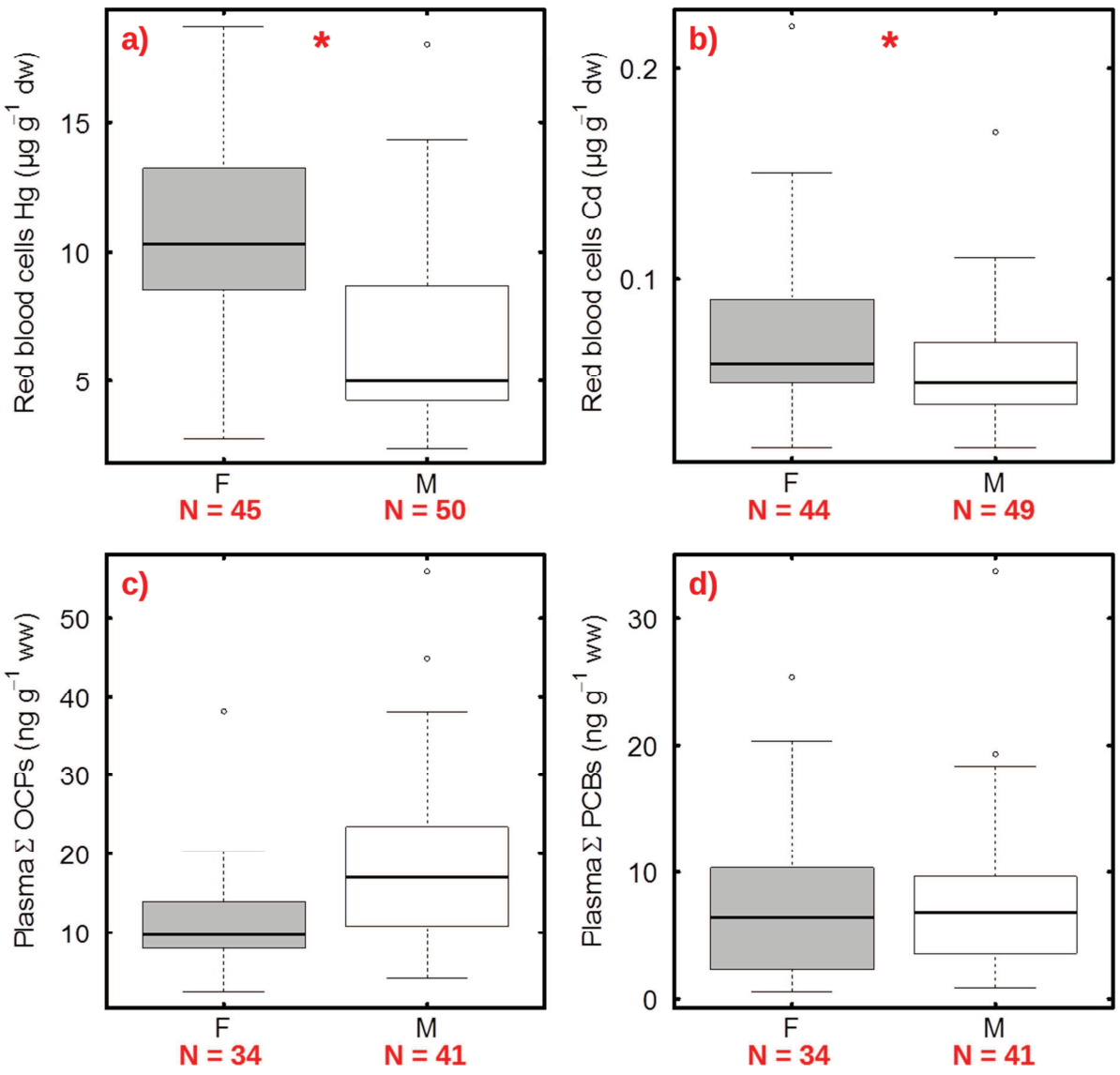

Fig. 10. *Box plots of blood* ***a)*** *Hg,* ***b)*** *Cd,* ***c)*** *Σ_{11}OCPs and* ***d)*** *Σ_7PCBs concentrations in female and male breeding wandering albatrosses from the Crozet Islands. OCPs: cis-chlordane, trans-nonachlor, HCB, γ-HCH, heptachlor, mirex, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT. PCBs: CB-28/50, -52, -101, -118, -138, -153 and -180. The asterisks indicate a significant difference, tested statistically through multi-factorial LMs (Hg) and GLMs (Cd and POPs) including other variables, such as age and feeding habitat ($\delta^{13}C$) (more details in the next sections and in Paper 4). Box plots show the median (horizontal line within the box), 1st and 3rd quartiles (lower and upper margins), and 1st – 1.5 x interquartile range 3rd+1.5 x interquartile range (lower and upper whiskers), and outliers. Abbreviations: F, females; M, males. Data from* ***Paper 4****.*

Results and discussion. Unexpectedly, females had significantly higher blood Hg concentrations than males (Fig. 10). This pattern has already been shown in the wandering albatross (Hindell et al. 1999, Tavares et al. 2013) and other seabirds (Monteiro and Furness 2001, Becker et al. 2002, Gonzáles-Solís et al. 2002), and indicates that the amount of Hg

excreted in the egg is likely negligible when compared to the total body burden. Furthermore, steady state equilibrium with dietary uptake of Hg likely compensates rapidly for the amount lost in the egg (within weeks, Furness and Greenwood 1993). Female has also higher blood concentrations of Cd, but the sex difference had poor explanatory power (the GLM: Cd ~ sex, which had the best fit to the Cd data, explained only 5% of the total variation). No sexual differences were detected for POPs, even if there was a tendency for females to show lower OCPs concentrations than males (Fig. 10). Sequestration in the lipid-rich egg may potentially be more important for some POPs than for Hg in the wandering albatross. However, POPs egg transfer has proved to be significant only for species with large egg clutches (*e.g.*, Drouillard and Norstrom 2000, Robinson et al. 2012), whereas the wandering albatross lays only one egg at each breeding attempt (Tickell 1968). The sex-related variation in feeding strategies of wandering albatrosses during the breeding season could explain the sexual pattern highlighted here in blood environmental contaminant concentrations (see section 3.5.2.). Thus, gender seems to be of interpretive concern only for species exhibiting different dietary habits between sexes (Gonzáles-Solís et al. 2002, Evers et al. 2005, Robinson et al. 2012). Nevertheless, it cannot be excluded that other physiological traits are involved, such as different Me-Hg demethylation capabilities between males and females (Robinson et al. 2011).

Conclusions. Egg transfer alone is not critical in explaining sexual differences in environmental contaminant concentrations, in particular when compared to feeding strategies. Sampling either males or females could imply bias in biomonitoring studies only in species with sex-related differences in dietary habits and/or marked sexual size dimorphism.

3.4.2. Reproductive status

Individual seabirds that are not engaged in reproduction may be difficult to sample and in some instances to recognize (Mallory et al. 2006). In this doctoral work, the long-term survey on the wandering albatross on the Crozet Islands has offered a unique opportunity to study contaminant burdens at different breeding stages, since the individual breeding history was known for a large number of birds.

State of the art and hypothesis. Since birds at different breeding stages may have different physiological traits (*e.g.*, hormone and lipids dynamics and metabolic rates), energy requirements (*e.g.*, Cherel et al. 1994b, Salamolard and Weimerskirch 1993, Borgå et al. 2004), and feeding habits (Jaeger et al. 2014), they could show different environmental contaminant concentrations. However, the influence of reproductive status on contaminant concentrations has received little attention. Some studies have compared breeding and immature birds (Eagles-Smith et al. 2009, Tavares et al. 2013), while others have sampled adult non-breeding individuals (Van den Brink et al. 1998, Mallory et al. 2006, 2007). I therefore decided to evaluate the breeding status effect on environmental contaminant concentrations in three groups: **immature** (pre-breeding), **breeding** and **non-breeding** mature individuals. In previous studies, breeding status differences were usually not significant for POPs concentrations, especially in males. Conversely, surprisingly higher feather Hg concentrations were detected in immature wandering albatrosses at South Georgia, southern Atlantic Ocean (Tavares et al. 2013). *Hence, I expected higher concentrations of Hg in immature than in non-breeding and breeding individuals, while blood POPs concentrations were expected to be similar between groups*. Because of unbalanced sample sizes in the female group (only two non-breeding females were sampled), the effect of breeding status was investigated in **males** only.

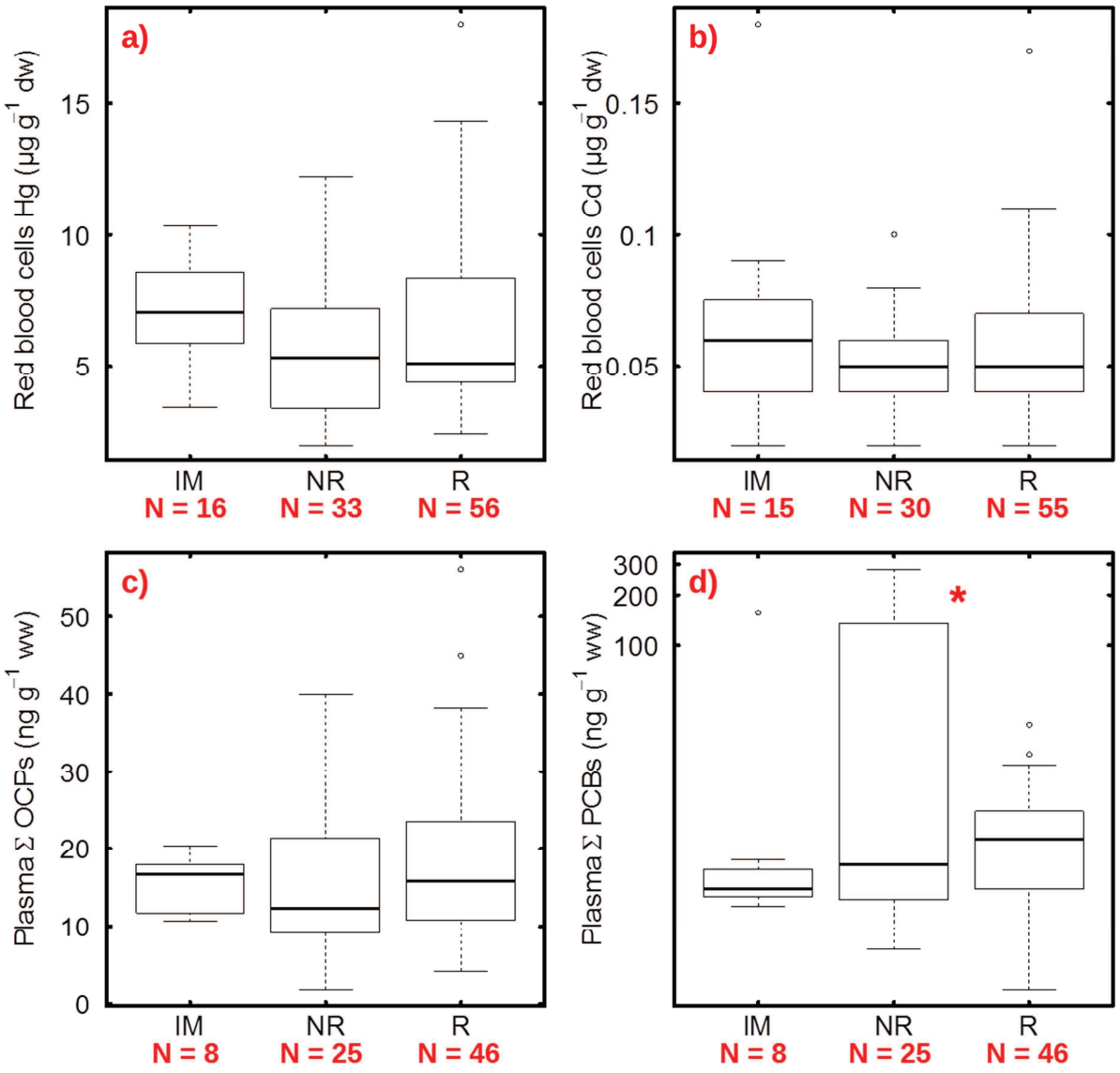

Fig. 11. *Blood **a)** Hg, **b)** Cd, **c)** Σ_{11}OCPs and **d)** Σ_7PCBs (on a logarithmic scale) concentrations in **male** wandering albatrosses of different breeding status. OCPs: cis-chlordane, trans-nonachlor, HCB, γ-HCH, heptachlor, mirex, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT. PCBs: CB-28/50, -52, -101, -118, -138, -153 and -180. The effect of breeding status was statistically tested through unifactorial LMs (Hg) and GLMs (Cd, Σ_{11}OCPs, and log-transformed Σ_7PCBs). Breeding status had a significant effect only on Σ_7PCBs (taking into account the plasma lipid content, $p = 0.014$, $N = 79$). Abbreviations: IM, immature; NR, non-breeding; R, breeding individuals. Data from **Paper 4**.*

Results and discussion. As expected, blood environmental contaminant concentrations were not significantly influenced by the breeding status in males (Fig. 11). Blood PCBs concentrations showed very high within-individual variation in non-breeding individuals, which calls for caution in interpretation. **Immature** birds tended to have higher Hg concentrations than breeders and non-breeders, but the difference was clearly not

significant. This contrasts with results on feathers (Tavares et al. 2013 and Polartop unpublished data), possibly because blood Hg represents exposure over a shorter period than feathers. Immature wandering albatrosses were shown to have fewer new flight feathers at the colony than did breeding individuals (Weimerskirch 1991). If immature birds moulted less frequently, they would have less opportunities to excrete Hg than adults (Tavares et al. 2013), but there is no conclusive evidence to support this explanation for a higher Hg burden in immatures.

Conclusions. While further investigations are highly needed in order to understand the potential influence of breeding status on contaminant concentrations, especially in females, males in different reproductive stages did not show large differences in blood environmental contaminant concentrations. This has an interesting implication for biomonitoring, because the difficult breeding status evaluation (for example for seabirds sampled far from their breeding colonies) can be avoided, without biasing long-term monitoring studies.

3.4.3. Phylogeny

Phylogeny-specific physiological traits may lead to differential accumulation and/or detoxification of environmental contaminants in seabirds. In my doctoral work, I could look at potential differences in feather Hg concentrations between crude taxonomic groups of the Kerguelen avian community. This community offers an excellent study system of phylogeny-related variation in contaminant concentrations because it encompasses a high number of closely-related species (ten families from five orders: Sphenisciformes (penguins), Procellariiformes (albatrosses and petrels), Charadriiformes (skuas and gulls), Suliformes (cormorants) and Anseriformes (ducks), see Table 3, Chapter 1).

State of the art and hypothesis. Inter-specific differences in avian Hg exposure and accumulation have been often investigated, but taxonomic-related variations were rarely

tested in a large number of species (*e.g.*, Stewart et al. 1999, Anderson et al. 2009, Ochoa-Acuña et al. 2002). These studies have shown that phylogeny has not a determinant role in explaining between-species differences when compared to ecological factors. Thus, *I expected that taxonomic groups should show weak explanatory power of feather Hg concentrations in adult birds from the Kerguelen community*. However, some taxonomic groups have received little attention (*e.g.*, the gadfly petrels). Hence, potential group-specific patterns were not excluded.

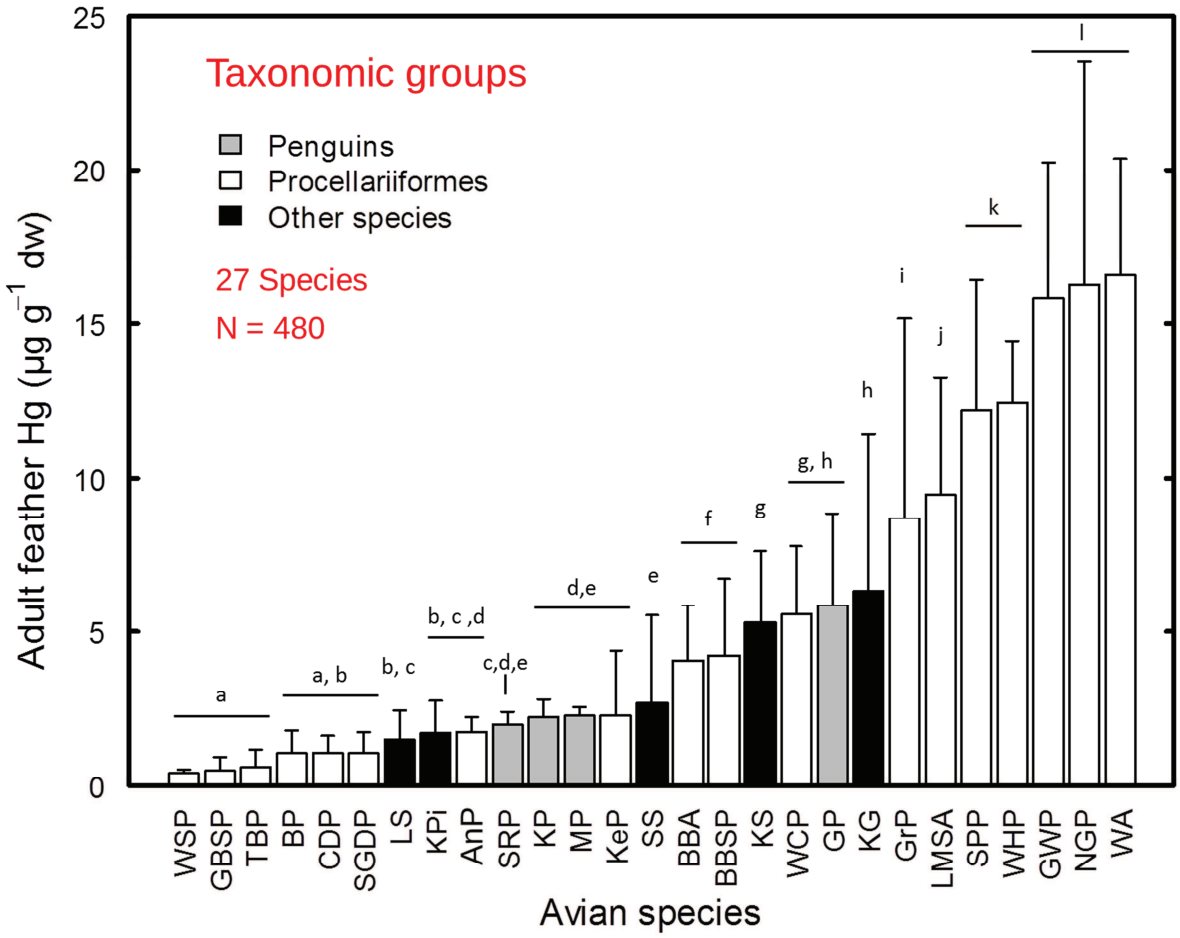

Fig. 12. *Bar plot of adult feather Hg concentrations within the Kerguelen avian community. Species are presented according to their taxonomic groups: penguins (grey), procellariiform seabirds (white) and other species (black). Species sharing the same letter are not statistically different (Tukey Honest Significant Difference, HSD, $p < 0.05$). Values are means + SD. Abbreviations: WSP, Wilson's storm petrel; GBSP, grey-backed storm petrel; TBP, thin-billed prion; BP, blue petrel; CDP, common diving petrel; SGDP, South Georgian diving petrel; LS, lesser sheathbill; KPi, Kerguelen pintail; AnP, Antarctic prion; SRP, southern rockhopper penguin; KP, king penguin; MP, macaroni penguin; KeP, Kerguelen petrel; SS, subantarctic skua; BBA, black-browed albatross; BBSP, black-bellied storm petrel; KS, Kerguelen shag; WCP, white-chinned petrel; GP, gentoo penguin; KG, kelp gull; GrP, grey petrel; LMSA, light-mantled sooty albatross; SPP, soft-plumaged petrel; WHP, white-headed petrel; GWP, great-winged petrel; NGP, northern giant petrel; WA, wandering albatross. Modified from Fig.2 in Paper 3.*

Results and discussion. Strong between-species differences were detected in feather Hg concentrations in the Kerguelen community (Fig. 12), with a factor of 40 between the species with the lowest and the highest mean values. **Species** was the best taxonomic explanatory variable compared to genus, family or order, which had poor explanatory power (for more details see Table 1 in **Paper 3**) This result was expected, since the species integrates a large range of ecological, behavioural, physiological and life-history traits that are susceptible to drive variation in feather Hg concentrations (Anderson et al. 2009, Bond and Diamond 2009b). Indeed, closely-related species at Kerguelen often showed very different levels of exposure. For example, black-bellied storm petrels displayed higher Hg concentrations than the other two storm petrel species, despite similar size and life-history traits. The same pattern was highlighted for penguins, with the gentoo penguins having higher feather Hg concentrations than the other three penguin species (Fig. 12). Nevertheless, new and interesting results were highlighted in a particular taxonomic group: the **gadfly petrels** (genus *Pterodoma*, Warham 1990), which were amongst the species with the highest Hg concentrations (Fig. 12), as confirmed also by previous studies on this genus (Thompson et al. 1990, 1993, Ochoa-Acuña et al. 2002). This could be linked to several non-exclusive factors, such as high Hg absorption rates, high Hg feather excretory capacities and/or high exposure from the diet. Indeed, between-species differences seemed to be explained by known feeding habits of the species (see next section).

Conclusion. The effect of taxonomy seems to play a minor role in avian Hg exposure and accumulation when compared to other ecological factors. Nevertheless, some groups such as the gadfly petrels merit further investigation.

3.5. Influence of extrinsic factors: does feeding ecology explain contaminant exposure?

Given the biomagnification phenomenon observed for some environmental contaminants in particular Hg and POPs, trophic position is recognized to be a critical factor in environmental contaminant exposure and accumulation in vertebrates. While links between environmental contaminant levels and trophic position are sometimes assessed through a broad scale approach in seabirds (Stewart et al. 1997, Thompson et al. 1998b, Sagerup et al. 2002), several studies have shown significant relationships between trophic tracers and contaminant concentrations, especially in food webs and species communities (*e.g.*, Atwell et al. 1998, Fisk et al. 2001a,b, Bond and Diamond 2008).

3.5.1. Between-species variation in Hg exposure: case study of the Kerguelen community

The Kerguelen community comprises species with contrasting feeding strategies that are relatively well-known during the breeding period (Table 3, Chapter 1). In order to study the influence of feeding habits on environmental contaminant exposure in TAAF birds, my doctoral work has thus focussed largely on this community (**Papers 2, 3 and 5**).

State of the art and hypotheses. Since 1) Hg is the only trace element in which there is clear evidence for biomagnification up food webs (*e.g.*, Atwell et al. 1998, Monteiro et al. 1998), and 2) diet is believed to be a critical driver of Hg exposure in seabirds (*e.g.*, Becker et al. 2002, Anderson et al. 2009), it seems likely that *avian species with increasing trophic positions would show increasing Hg concentrations in their tissues*. I tested this hypothesis in feathers through two approaches. **First**, I used the **isotopic niche** as a proxy of the trophic niche in **chicks**, since contaminants and stable isotopes are integrated over the same period in their feathers (see Chapter 2). **Second**, I exploited previous knowledge on feeding habits to

define different broad **dietary groups** in **adults,** since in their feathers there is temporal uncoupling of stable isotope and Hg integration (see Chapter 5). Avian species from the Kerguelen community feed on crustaceans, fish, squids, birds and carrion (Table 3, Chapter 1), and thus encompass different trophic levels. Hg contents of pelagic organisms from the Southern Ocean (including Kerguelen Islands) increase in the order crustaceans < fish ≤ squids < seabirds (Bustamante et al. 2003, Bocher et al. 2003, Anderson et al. 2009). *Hence, I expected that feather Hg concentrations would increase with $\delta^{15}N$ values in chicks and with dietary groups of increasing trophic level in adults.*

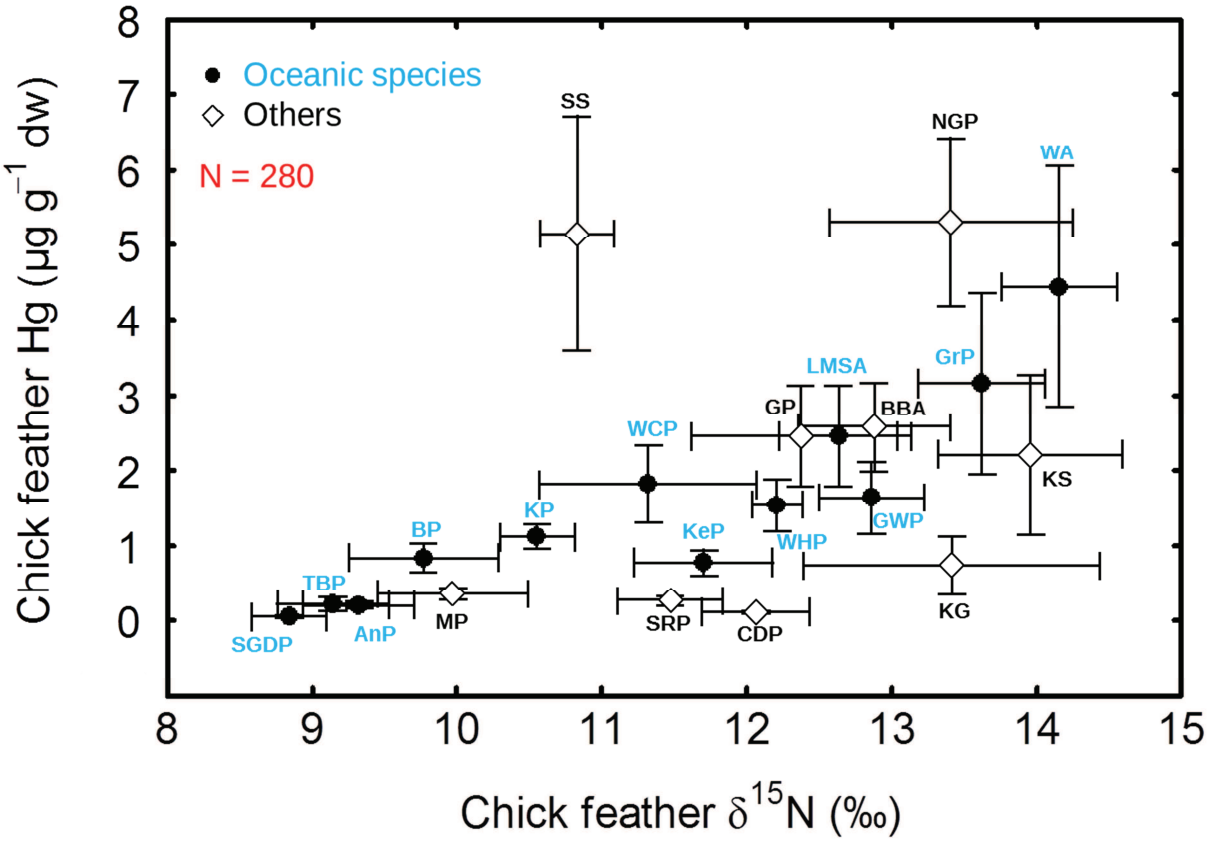

Fig. 13. *Correlation between chick feather Hg concentrations (means ± SD) and trophic position (chick feather $\delta^{15}N$) (Pearson correlation, r = 0.48, t = 9.19, p < 0.001, on individual values: N = 280). Filled circles and empty diamonds refer to oceanic and other species, respectively. Abbreviations: **oceanic species**: SGDP, South Georgian diving petrel; TBP, thin-billed prion; AnP, Antarctic prion; BP, blue petrel; KP, king penguin; WCP, white-chinned petrel; KeP, Kerguelen petrel; WHP, white-headed petrel; LMSA, light-mantled sooty albatross; GWP, great-winged petrel; GrP, grey petrel; WA, wandering albatross; **other species**: MP, macaroni penguin; SS, subantarctic skua; SRP, southern rockhopper penguin; CDP, common diving petrel; GP, gentoo penguin; BBA, black-browed albatross; KG, kelp gull; NGP, northern giant petrel; KS, Kerguelen shag. Modifed from Fig. 2 in Paper 5.*

Results and discussion. Kerguelen seabird **chicks** showed a wide range of feather Hg concentrations, with a ~100-fold difference between the species with the lowest and highest values (0.05 ± 0.01 to 5.3 ± 1.1 µg g^{-1} dw, mean ± SD, in South Georgian diving petrels *Pelecanoides georgicus* and northern giant petrels, respectively). As expected, feather Hg concentrations were positively related to $\delta^{15}N$ values (Fig. 13), *i.e.* to the chicks' trophic positions. Noticeably, the correlation was partially hindered by pooling species that forage in distinct habitats (neritic *vs.* oceanic and benthic *vs.* pelagic) marked by **different isotopic baselines** (see section 2.4.). Within this context, the positive correlation between Hg and $\delta^{15}N$ is particularly relevant. Indeed, the relationship was even stronger when looking at **oceanic seabirds** only (12 species, black circles, Fig. 13), *i.e.* when focussing on a unique isotopic baseline. These results therefore indicate that Hg strongly **biomagnifies** in subantarctic waters of the southern Indian Ocean, resulting in increasing Hg concentrations in the order crustacean- < fish- ≤ squid- < seabird/carrion-eating seabirds. The subantarctic skua was clearly an outlier species within the Kerguelen seabird assemblage, with chick Hg concentration being disproportionately high when compared to their feather $\delta^{15}N$ (Fig. 13). As mentioned before (section 3.3.1.), skua chicks are fed almost exclusively with the flesh of small seabirds (*i.e.* mainly the *viscus*), in particular of blue petrels (Mougeot et al. 1998), which contain disproportionately more Hg than fish and crustaceans (Bocher et al. 2003, Bustamante et al. 2003). Indeed, individuals feeding predominantly on other seabirds contain more Hg than conspecifics feeding on fish, as shown in the Antarctic skua *Catharacta maccormicki* (Goutte et al. 2014b) and in the great skua *Chataracta skua* from the Northern Hemisphere (Bearhop et al. 2000a,b).

Adults also displayed a wide range of Hg exposures (from 0.4 ± 0.1 to 16.6 ± 3.8 µg g^{-1} dw, mean ± SD, in Wilson storm petrels *Oceanites oceanicus* and wandering albatrosses, respectively, *i.e.* a ~40-fold difference), with their concentrations being significantly higher

than those of chicks (see section 3.3.1.). The pattern of between-species differences in Hg concentrations was similar to that of chicks: terrestrial species and crustacean-eaters, such as small petrels and penguins, generally showed low concentrations (< 2.5 µg g^{-1} dw); coastal seabirds, large petrels and small albatrosses, which feed extensively on fish, had intermediate concentrations (< 10 µg g^{-1} dw), while **gadfly petrels**, northern giant petrels and wandering albatrosses, which rely largely on squids and carrion, were the species having the highest concentrations (> 10 µg g^{-1} dw). Therefore, categorisation by dietary groups (Fig. 14) of adult species fairly explained feather Hg concentrations better than the taxonomic classification (Fig. 12). These results in feathers are consistent with previous works on different tissues of birds from other subantarctic avian communities (southern Atlantic Ocean: Muirhead and Furness 1988, Becker et al. 2002, Anderson et al. 2009; southern Pacific Ocean: Lock et al. 1992, Stewart et al. 1999).

Subantarctic skuas fighting for a small petrel on the Kerguelen Islands (photoThibaut Lacombe).

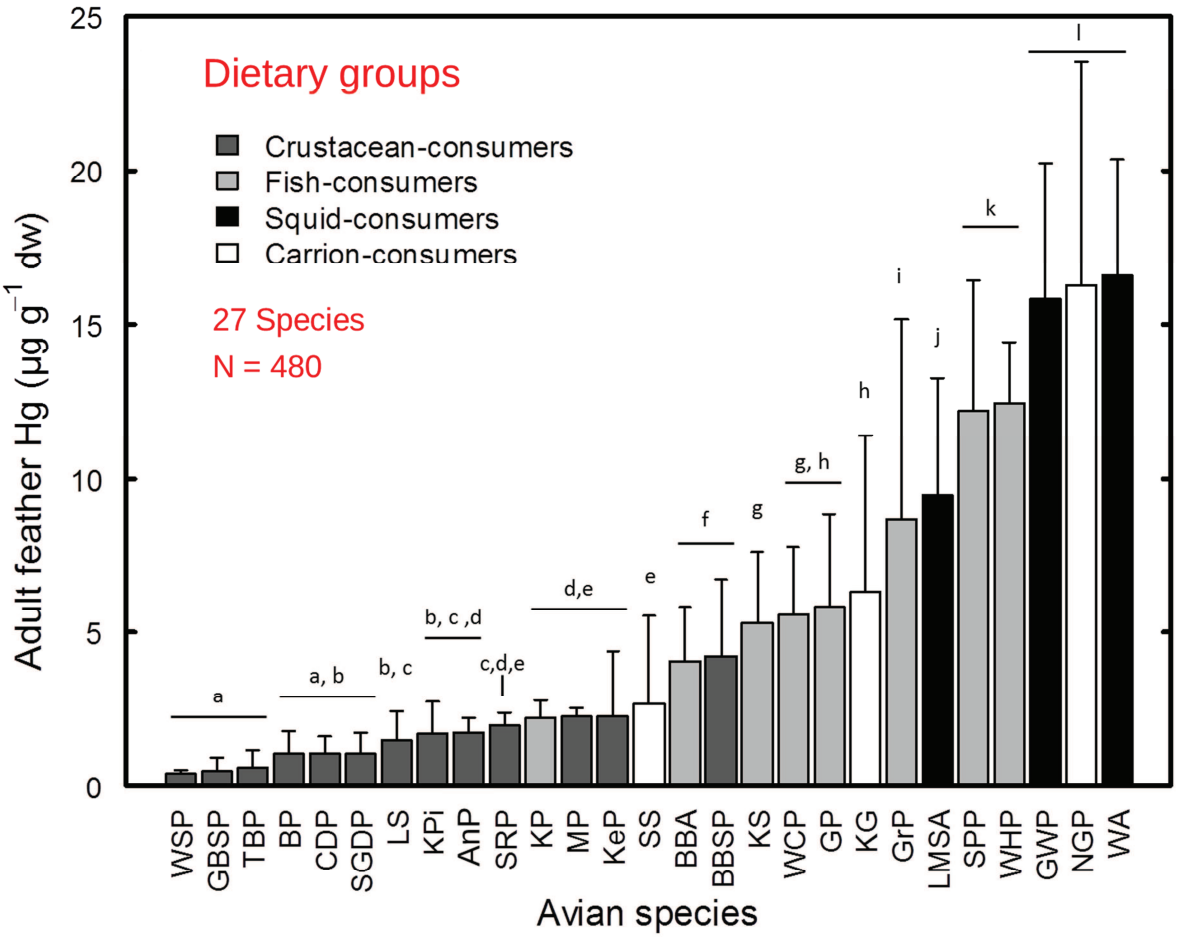

Fig. 14. *Bar plot of adult feather Hg concentrations within the Kerguelen avian community. Species are presented according to their dietary groups: terrestrial species and crustacean- (dark grey), fish- (grey), squid- (black) and carrion- (white) consumers. Note that subantarctic skuas were considered carrion-eaters based on chick diet, because knowledge on adult feeding habits outside the breeding period is too limited to conclude on a lower trophic-level diet. Species sharing the same letter are not statistically different (Tukey HSD, $p < 0.05$). Values are means + SD. Abbreviations: WSP, Wilson's storm petrel; GBSP, grey-backed storm petrel; TBP, thin-billed prion; BP, blue petrel; CDP, common diving petrel; SGDP, South Georgian diving petrel; LS, lesser sheathbill; KPi, Kerguelen pintail; AnP, Antarctic prion; SRP, southern rockhopper penguin; KP, king penguin; MP, macaroni penguin; KeP, Kerguelen petrel; SS, subantarctic skua; BBA, black-browed albatross; BBSP, black-bellied storm petrel; KS, Kerguelen shag; WCP, white-chinned petrel; GP, gentoo penguin; KG, kelp gull; GrP, grey petrel; LMSA, light-mantled sooty albatross; SPP, soft-plumaged petrel; WHP, white-headed petrel; GWP, great-winged petrel; NGP, northern giant petrel; WA, wandering albatross. Modified from Fig. 2 in Paper 3.*

Conclusions: Trophic positions had a critical role in explaining Hg exposure of both chicks and adults of Kerguelen avian species. This highlights the occurrence of Hg biomagnification processes in the Southern Ocean. Trophic position should thus be determined and accounted for in long-term biomonitoring studies.

3.5.2. Between-individual variation in contaminant exposure: case study of gentoo penguins and wandering albatrosses

State of the art. By studying between-species differences in feather Hg concentrations, an important point emerged from my results: **between-individual variation** was highly variable depending on species, as shown by the large SD in some adult feather Hg concentrations (Fig. 14). It is a usual pattern to observe increasing between-individual variability in species with high contaminant concentrations, such as the northern giant petrel or the wandering albatross. However, species with intermediate concentrations within the Kerguelen community, such as the kelp gull *Larus dominicanus* or the gentoo penguin, also showed high variation between individuals. Causes of intraspecific variation in environmental contaminant exposure and accumulation are less well characterised than between-species differences (Hipfner et al. 2011). Several studies have however shown that foraging strategies play an important role also in explaining differences in concentrations between individuals (*e.g.*, Bearhop et al. 2000a,b, Bustnes et al. 2000, Steffen et al. 2006), but only few have succeed in linking observed concentrations and trophic tracers within a single species (Bearhop et al. 2000a, Sagerup et al. 2002, Ramos et al. 2009). Different foraging strategies can result from feeding on different prey in the same habitat, but also foraging in different habitats (and hence feeding on different prey). In my doctoral work, I investigated the influence of dietary habits on between-individual variation in environmental contaminant concentrations in two steps: **first** at a **small geographical scale**, by comparing two sub-populations of the short-ranging neritic gentoo penguins at the Kerguelen Islands (**Paper 2**) and, **second** at a **wide geographical scale** by using the large data set on the wide-ranging oceanic wandering albatross from the Crozet Islands (**Paper 4**).

3.5.2.1. Gentoo penguins at the Kerguelen Islands

Hypothesis. Gentoo penguins are neritic opportunistic feeders. They rely mainly on crustaceans and fish, with their diet composition varying greatly both with the locality and the breeding colony (Bost and Jouventin 1990, Lescroël et al. 2004). At the Kerguelen Islands gentoo penguins are present year round (resident species) and forage under contrasting oceanographic conditions (Lescroël and Bost 2005). In the context of the Polartop project, gentoo penguins were sampled at two breeding sites (Fig. 15a): **Penn Island**, where penguins forage in the surrounding **closed Morbihan Bay** (diet mainly based on pelagic crustaceans), and **Cape Estacade**, where individuals forage in the **open ocean** environment (diet mainly based on benthic fish). Since these two sub-populations show different feeding strategies (Lescroël and Bost 2005), *I expected individuals relying more largely on benthic fish (higher $\delta^{15}N$) to have higher Hg concentrations in their feathers than conspecifics relying mainly on pelagic crustaceans (lower $\delta^{15}N$).*

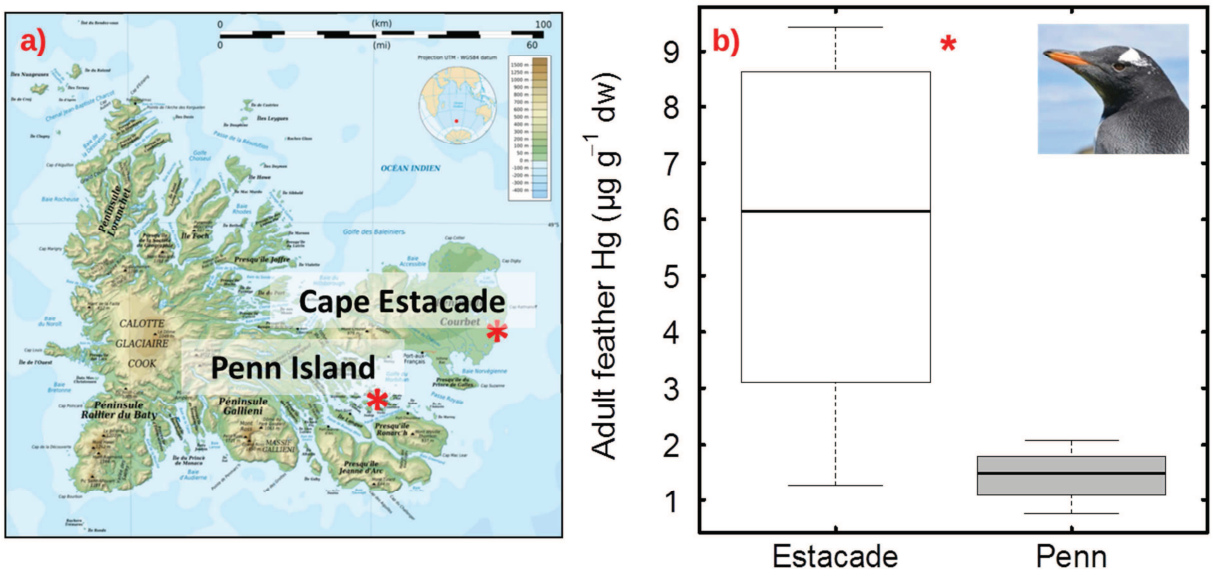

Fig. 15. ***a)*** *Penn Island and Cape Estacade position at the Kerguelen Islands and* ***b)*** *box plot of feather Hg concentrations in the two sub-populations of gentoo penguins, which were significantly different (Wilcoxon, $W = 137$, $p < 0.0001$, $N = 24$). Data from* ***Paper 2****.*

Results and discussion. **Cape Estacade** gentoo penguins displayed on average four times higher feather Hg concentrations than their **Penn Island** conspecifics (5.9 ± 3.0 and 1.4

± 0.4 µg g^{-1} dw, mean ± SD, respectively, Fig. 15b). Such striking differences in feather Hg concentrations within the same species seem difficult to attribute to physiological factors. Instead, they could be related to diet preferences. Penn Island individuals feed extensively on swarming **pelagic crustaceans** (85% of the diet by mass at different sites of the bay; Lescroël et al. 2004). On the other hand, Cape Estacade individuals present a more diversified diet, including a large proportion of **benthic fish**, but also pelagic crustaceans (71% and 13% of the overall diet by mass, respectively; Lescroël et al. 2004). Accordingly, they presented low and high feather Hg concentrations, consistent with species feeding on crustaceans and fish, respectively (Becker et al. 2002, Bocher et al. 2003, Stewart et al. 1999 and section 3.5.1). Furthermore, benthic organisms are known to bioaccumulate high quantities of Hg when compared to epipelagic ones (Bustamante et al. 2006, Storelli et al. 2005, Cossa and Gobeil 2000). Importantly, Cape Estacade gentoo penguins had high between-individual variation in feather Hg concentrations that were, as expected, significantly and positively correlated to stable isotopes (Fig. 16). This indicates a succession of **specialised foraging individuals**, ranging from birds feeding almost exclusively on pelagic crustaceans (reflected by low feather $\delta^{13}C$ and $\delta^{15}N$ values) to birds feeding almost exclusively on benthic fish (high feather $\delta^{13}C$ and $\delta^{15}N$ values), therefore showing low to high feather Hg concentrations. Conversely, Penn Island penguins showed low between-individual variation in feather Hg concentrations, since they specialised on small pelagic crustaceans, which are very abundant in the Morbihan Bay (Bocher et al. 2001).

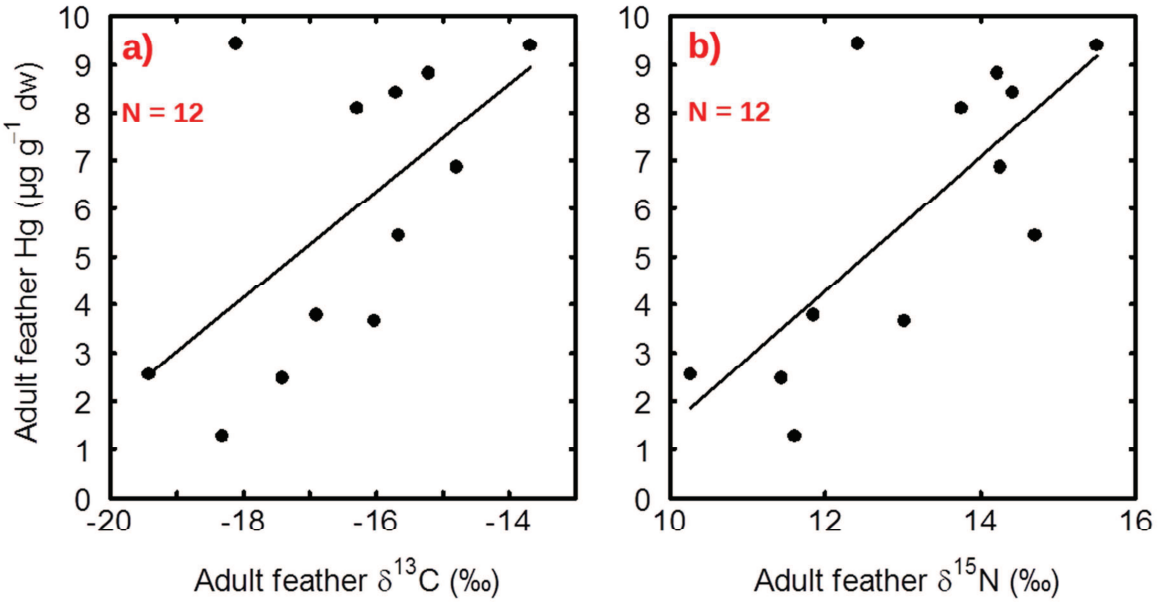

Fig. 16. *Relationships between feather Hg concentrations and* ***a)*** *foraging habitat (adult feather* $\delta^{13}C$*) and* ***b)*** *trophic position (adult feather* $\delta^{15}N$*) in gentoo penguins from Cape Estacade. The correlations are highly significant (Pearson correlation, r = 0.61 and 0.74 for* $\delta^{13}C$ *and* $\delta^{15}N$*, respectively, p < 0.05 for both). Modified from Fig. 3 in* ***Paper 2****.*

Conclusion. **Individual specialisation** of feeding strategies, including both trophic position and foraging habitat, plays a critical role in driving variation in Hg exposure in the gentoo penguin, and need to be evaluated for other species. High variability in Hg concentrations could blur spatio-temporal trends in long-term studies of environmental contamination. Hence, **populations** with a **variable diet** are less suitable as bioindicators of environmental contamination than populations with a **specialised diet**.

3.5.2.2. Wandering albatrosses from the Crozet Islands

State of the art and hypothesis. The wandering albatross is a wide-ranging species, with exceptional flight capacities. During the breeding season, birds from the Crozet Islands can travel up to 3500 km far from their nest, with sex-related differences in feeding habitats: males forage in subantarctic and Antarctic waters, while females forage in warmer waters north of the archipelago (*e.g.*, Jaeger et al. 2010a, Weimerskirch et al. 2014 and section 3.2.).

Foraging over such a wide range of environments was expected to result in high variations of exposure to environmental contaminants, which distribution is heterogeneous in the marine environment (see Chapter 1). In order to avoid repetitions, the hypotheses and predictions concerning the geographical distribution of environmental contaminants will be detailed in the next chapter.

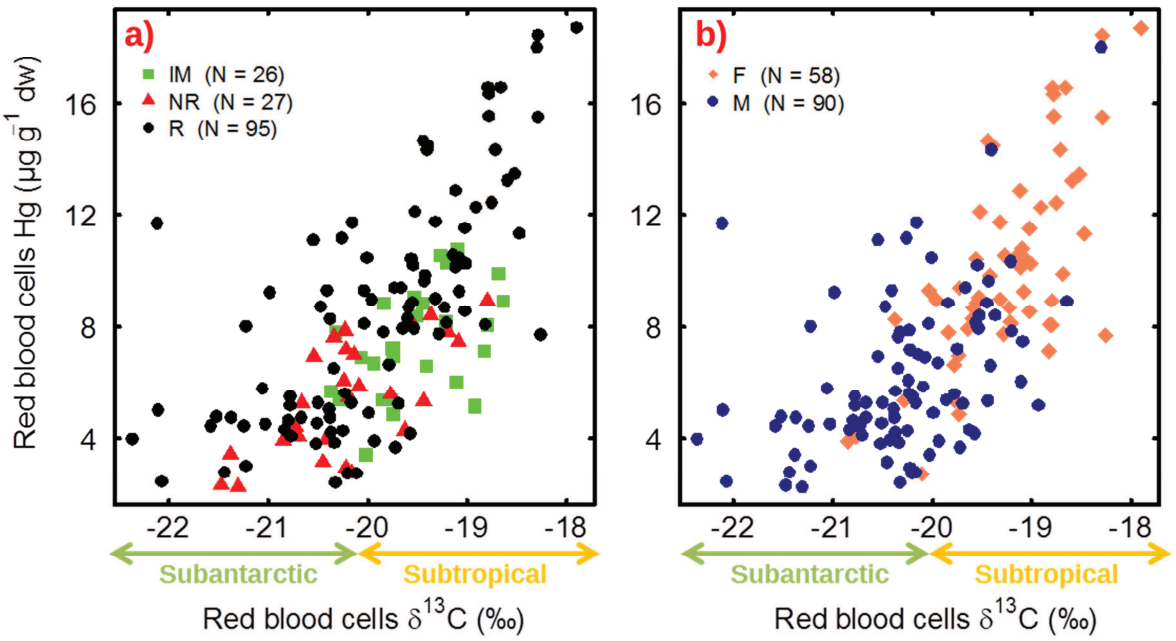

Fig. 17. *Relationships between blood Hg concentrations and foraging habitat (blood $\delta^{13}C$) depending on* ***a)*** ***breeding status*** *and* ***b)*** ***sex*** *in wandering albatrosses from the Crozet Islands. Multi-factorial statistical analyses were realised on breeding individuals only (R, black points), with the best model explaining Hg concentrations being Hg = $\delta^{13}C$ + sex + $\delta^{13}C$*sex (LM, for more details see Paper 4). Abbreviations: IM, immature; NR, non-breeding; R, breeding individuals; F, females; M, males. Data from* ***Paper 4****.*

Results and discussion. As expected, wandering albatrosses showed high between-individual differences in blood contaminant concentrations (for example, coefficients of variation were often higher than 100% for POPs; for more details see Supplementary Material of **Paper 4**). Blood Hg concentrations were strongly and positively related to $\delta^{13}C$ values, and hence to feeding habitat (Fig. 17). Namely, individuals with increasing blood Hg concentrations had increasing blood $\delta^{13}C$ values, *i.e.* foraged at decreasing latitudes of the southern Indian Ocean. The strong relationship was verified at the **population level**, **irrespective of breeding status** (Fig. 17a) **or sex** (Fig. 17b). More precisely, females and

immature birds, foraging mainly in warmer subtropical waters (high $\delta^{13}C$ values), showed higher Hg concentrations than males, which foraged in colder high subantarctic waters (low $\delta^{13}C$ values). Hence, it seems likely that the sexual difference in blood Hg concentrations was related to dietary habits rather than intrinsic physiological characteristics (section 3.4.1.). To the best of my knowledge, no previous studies have shown such a strong relationship between Hg and foraging strategies within a single seabird population.

Similarly, blood OCPs concentrations were related to the foraging habitat ($\delta^{13}C$ values), although less strongly than Hg, with individuals foraging at high subantarctic latitudes (low $\delta^{13}C$ values) showing higher blood OCPs concentrations (Fig. 18). Again, the tendency of males to bear higher burdens of OCPs than females (section 3.4.1.) seems to be related to the sexual segregation in feeding strategies (Fig. 18b). This sexual difference had lower explanatory power than for Hg, probably because of the concurrence of several confounding factors. Indeed high unexplained variation in POPs concentrations is a common pattern in seabirds (*e.g.*, Sagerup et al. 2002, Colabuono et al. 2012). In particular, PCBs concentrations were so different between individuals that none of the tested explanatory variables explained satisfactorily the variation (except plasma lipid content to a certain extent, see **Paper 4**).

The influence of trophic level could not be directly tested, since there was a strong correlation between $\delta^{13}C$ and $\delta^{15}N$ values (LM, adjusted R squared, (R_{adj}^2) = 0.80, $p < 0.001$), related to the slight enrichment in baseline $\delta^{15}N$ values north of the subtropical front (Jaeger et al. 2010a). Over the large latitudinal gradient exploited by wandering albatrosses, the trophic-level information of $\delta^{15}N$ values is thus confounded by a feeding habitat effect

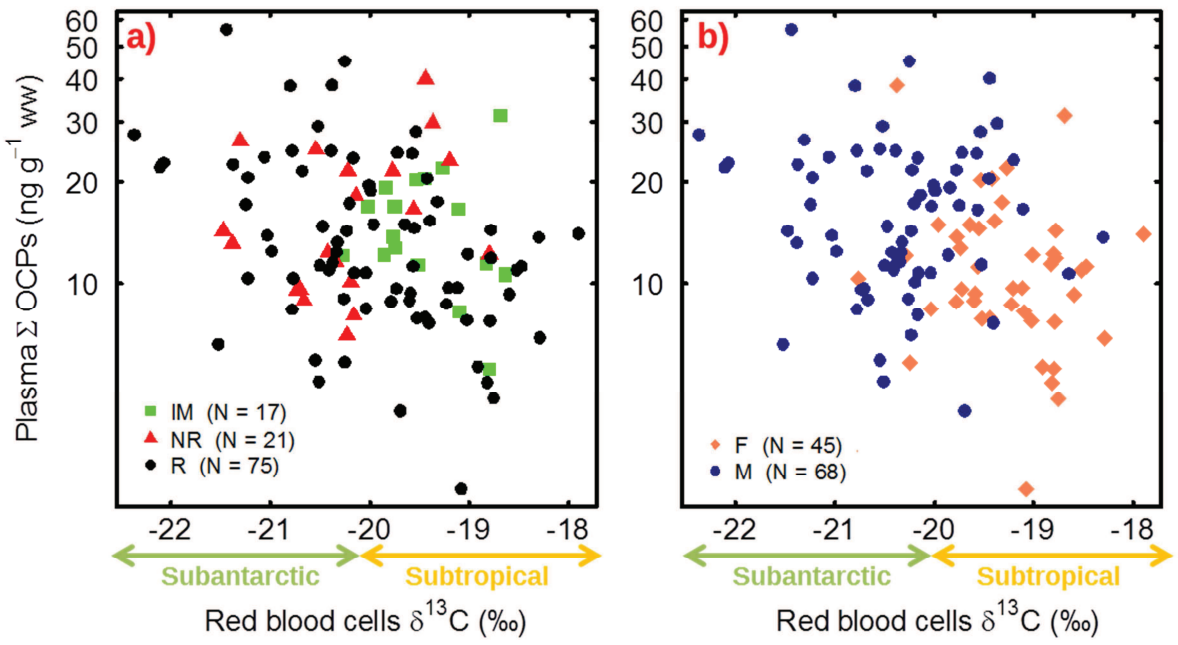

Fig. 18. *Relationships between blood Σ_{11}OCPs concentrations (on a logarithmic scale) and foraging habitat (blood δ^{13}C) depending on* ***a) breeding status*** *and* ***b) sex*** *in wandering albatrosses from the Crozet Islands. OCPs: cis-chlordane, trans-nonachlor, HCB, γ-HCH, heptachlor, mirex, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT. Multi-factorial statistical analyses were realised on breeding individuals only (R, black points), with the best model explaining Σ_{11}OCPs concentrations being Σ_{11}OCPs: δ^{13}C + sex (GLM, for more details see Paper 4). Abbreviations: IM, immature; NR, non-breeding; R, breeding individuals; F, females; M, males. Data from* ***Paper 4****.*

Conclusion. Individual specialisation of feeding strategies is critical in driving variation in Hg and POPs exposure in the wandering albatross. The high between-individual variability in contaminant concentrations may prevent the use of this species as an effective bioindicator. Nevertheless, its wide-ranging nature during the breeding period gives a unique opportunity to compare contaminant transfer over a large latitudinal scale from a single species and over a short time period. Careful assessment of individual foraging strategies (*i.e.* through the use of trophic tracers and/or direct techniques) could avoid misleading conclusions on environmental contaminant bioavailability in the environment.

3.6. Summary

The evaluation of intrinsic and extrinsic factors driving variation on seabird contaminant concentrations can be difficult in the wild. The case of the wandering albatross exemplifies the complexity of the underlying mechanisms and the utility of large sample sizes to understand multi-factorial interactions. The schematic and repetitive structure of this chapter reflects the need for considering the influence of each factor independently from the others before understanding its effect in a more complex context. Overall, results of this part of my doctoral work indicate that:

- **feeding ecology is the main factor driving variation in contaminant concentrations in seabirds, both at the community, sub-population and individual levels, and irrespective of age-class, sex, age and breeding status**;
- **age-class** is also an important intrinsic factor to consider, with chicks having a shorter time of exposure to contaminants than adults;
- **adult age, sex and phylogeny *per se*** have no significant effects on contaminant concentrations and thus would not, or weakly bias biomonitoring studies;
- seabird populations with a **specialised diet** are more reliable as biomonitors than populations with a **variable diet**; hence, **individual specialisation** should be carefully assessed before choosing bioindicator species of environmental contamination.

Chapter 4

Spatial and temporal variation in contaminant transfer to seabirds in the southern Indian Ocean

Macaroni penguins on the Kerguelen Islands
Photo Thibaut Lacombe

Little information is available on the distribution, trophic transfer and temporal trends of environmental contaminants in the Southern Ocean, as illustrated in Chapter 1. In order to infer potential spatial and temporal variations in contaminant concentrations in the southern Indian Ocean, I have used **adult penguins**, **chicks of different seabird species** and **adult wandering albatrosses** as bioindicators, as justified in the previous chapters. Here I focus specifically on Hg and POPs, because their geochemical cycles and trophic transfer are overall better understood than those of other environmental contaminants. This integrative chapter encompasses results from **Papers 4 and 5**, but also includes unpublished data that are still in the phase of **preliminary analyses**, and are reported in the form of tables in the Appendix.

4.1. Spatial trends of environmental contaminant transfer to seabirds

4.1.1. Spatial variation in Hg transfer to seabirds

State of the art and hypothesis. **Polar zones** have noticeable characteristics mainly in terms of light and temperature, making their Hg cycle unique. Peculiar processes are for example Hg depletion events (MDEs), first discovered in the Arctic (Schroeder et al. 1998), but occurring also in the Antarctic environment (Ebinghaus et al. 2002). These events take place during the polar springtime and consist in the rapid, photochemically driven formation of reactive inorganic Hg species in the atmosphere, which are deposited on snow surfaces. Despite consequent re-volatilisation from surfaces, MDEs seem to result in a significant net input of inorganic Hg to polar environments and to seawater through ice melting (Dommergue et al. 2010, Douglas et al. 2012). Moreover, the low temperature and ice cover reduce Hg re-emission from the water to the atmosphere, hence favouring the retention of Hg in polar marine environments (Douglas et al. 2012). High concentrations of Hg in Arctic biota have indeed been observed (*e.g.*, Muir et al. 1999, Braune et al. 2005, AMAP 2011).

However, studies have shown inconsistent latitudinal patterns in the Northern Hemisphere (*e.g.*, Douglas et al. 2012), with Hg concentrations at high subarctic and Arctic latitudes being higher (Braune et al. 2001, 2002), lower (Thompson et al. 1992a), or similar (Thompson et al. 1992b) to those at low latitudes, likely because geographical variation was confounded by the proximity to industrialized countries. In the Southern Hemisphere, high concentrations of atmospheric Hg have been observed close to the Antarctic continent when compared to lower latitudes (Soerensen et al. 2010). Moreover, the only geochemical investigation on Hg speciation and distribution in Southern Ocean waters has shown high concentrations of Me-Hg in Antarctic rather than subantarctic and subtropical waters (Cossa et al. 2011). *Hence, I expected that seabirds feeding in high-latitude water masses (Antarctic zone) would show higher Hg concentrations than those feeding in low-latitude waters (Subantarctic and Subtropical Zones)*. Blood and feather Hg concentrations from different species and different populations breeding on TAAF districts were compared by taking into account feeding ecology through stable isotopes and/or known dietary habits.

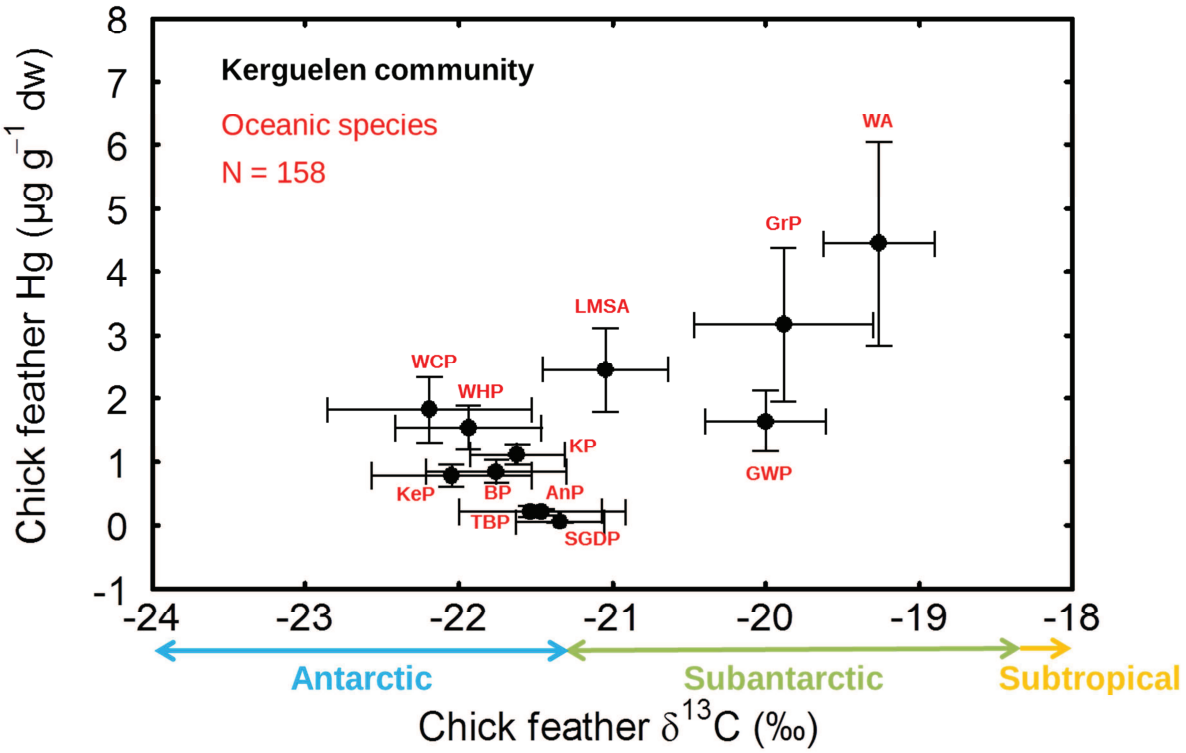

Fig. 19. *Correlation between chick feather Hg concentrations (means ± SD) and feeding habitat (chick feather $\delta^{13}C$) of **oceanic species** of the Kerguelen community (Pearson correlation, r = 0.61, t = 9.68, p < 0.001, on individual values: N = 158). Note that chick feather $\delta^{13}C$ values represent the habitats where their parents foraged during the chick-rearing period. Abbreviations: SGDP, South Georgian diving petrel; TBP, thin-billed prion; AnP, Antarctic prion; BP, blue petrel; KP, king penguin; WCP, white-chinned petrel; KeP, Kerguelen petrel; WHP, white-headed petrel; LMSA, light-mantled sooty albatross; GWP, great-winged petrel; GrP, grey petrel; WA, wandering albatross. Modifed from Fig. 2 in Paper 5.*

Results and interpretation. During the chick-rearing period, parent seabirds of the Kerguelen community rely on different habitats to capture prey for their chicks. Feather Hg concentrations of chicks that were fed with prey from Antarctic waters (low feather $\delta^{13}C$ values) were lower than those of chicks that were fed with prey from subantarctic waters (high feather $\delta^{13}C$ values) as shown in Fig. 19. This indirectly means that prey from subantarctic waters had higher Hg concentrations than prey from Antarctic waters.

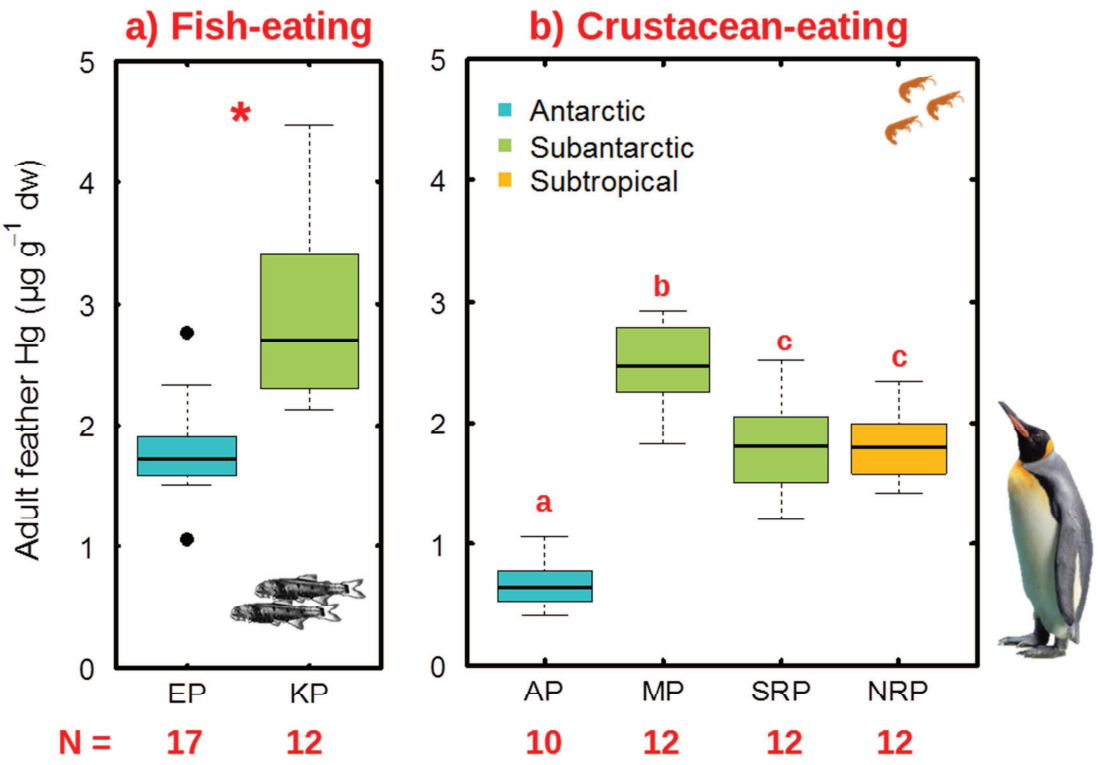

Fig. 20. *Box plots of adult feather Hg concentrations in **a)** mainly fish-eating and **b)** mainly crustacean-eating adult penguins, which are representative of different water masses in the southern Indian Ocean. Antarctic species were sampled at Terre Adélie, Antarctica; subantarctic species at Crozet Islands; the subtropical species at Amsterdam Island. The **a)** asterisk and **b)** different letters represent significant differences in feather Hg concentrations (Wilcoxon comparisons, all $p < 0.05$). The image on the right represents a king penguin. Abbreviations: EP, emperor penguin; KP, king penguin; AP, Adélie penguin; MP, macaroni penguin; SRP, southern rockhopper penguin; NRP, northern rockhopper penguin. Unpublished data, **Table A2** in the **Appendix**.*

Adult penguins have approximately constant feeding habits throughout their annual cycle. Namely, while they disperse more widely during the non-breeding than the breeding period, they exploit similar habitats, and are therefore representative of the same water masses all year long (Thiebot et al. 2011a,b, 2012, see Chapter 2). In the TAAF, Antarctic penguin species showed lower feather Hg concentrations than subantarctic and subtropical species, irrespective of the dietary group (fish-eating or crustacean-eating species, Fig. 20). For example, the crustacean-eating Adélie penguin *Pygoscelis adeliae*, which feeds in Antarctic waters year-round, had approximately four-times lower feather Hg concentrations than the subantarctic macaroni penguin *Eudyptes chrysolophus* (0.7 ± 0.2 *vs.* 2.5 ± 0.4 µg g^{-1} dw, mean ± SD, at Terre Adélie and Crozet Islands, respectively, see Table A2 in the Appendix

for more details). Macaroni and southern rockhopper penguins *Eudyptes chrysocome filholi*, the two crustacean-eating subantarctic species, had significantly different feather Hg concentrations, likely because macaroni penguins feed also on **mesopelagic fish** (Ridoux 1994), which may display high Hg content and a greater proportion of Me-Hg (*e.g.*, Bustamante et al. 2003, Choy et al. 2009, Chouvelon et al. 2012). Finally, the only subtropical species, the northern rockhopper penguin *Eudyptes chrysocome moseleyi*, had similar concentrations to those of the subantarctic closely-related southern rockhopper penguin. This could indicate that, at low levels of the food web (crustacean level), there was no increase in Hg concentrations from subantarctic to subtropical waters.

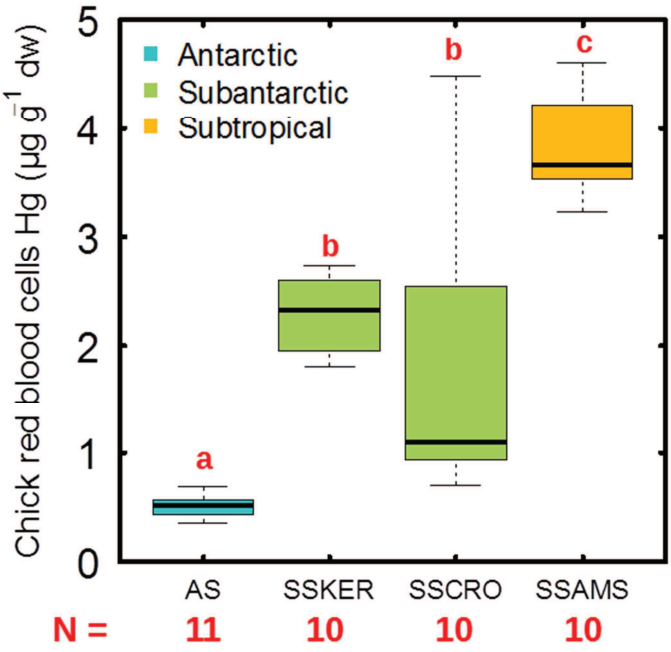

Fig. 21. *Box plots of chick blood Hg concentrations in Antarctic skuas from Terre Adélie (AS) and three populations of subantarctic skuas from the Kerguelen (SSKER), Crozet (SSCRO) and Amsterdam (SSAMS) Islands. Skua populations sharing the same letters have similar blood Hg concentrations (Wilcoxon comparisons, all p < 0.001). Unpublished data, **Table A3** in the **Appendix**.*

Skua species (*Catharacta* species) breed at the four TAAF districts, namely the Antarctic skua at Terre Adélie and the sibling species subantarctic skua at Kerguelen, Crozet and Amsterdam Islands. During the breeding period, the skua chick diet is largely composed of seabird meat (Table 3, Chapter 1). Skua chicks thus generally occupy a high trophic

position within their communities. Blood Hg concentrations increased from chicks of the Antarctic population to chicks of the subantarctic and subtropical populations (Fig. 21), with a factor of eight between the populations with the lowest and highest burdens (0.5 ± 0.1 *vs.* 4.0 ± 0.8 µg g^{-1} dw, mean ± SD, at Terre Adélie and Amsterdam Islands, respectively, see Table A3 in the Appendix for more details). **Between-population** comparisons thus indicate that the Hg exposure of high trophic-level seabirds increases from high to low latitudes in the southern Indian Ocean. This pattern is also confirmed at the **individual level** in the wandering albatross at a single locality. Indeed, feeding habitat had a strong explanatory power of blood Hg concentrations, with individuals feeding in northern subantarctic and in subtropical waters having higher Hg burdens than individuals feeding in southern colder waters (Fig. 22 and section 3.5.2.2.).

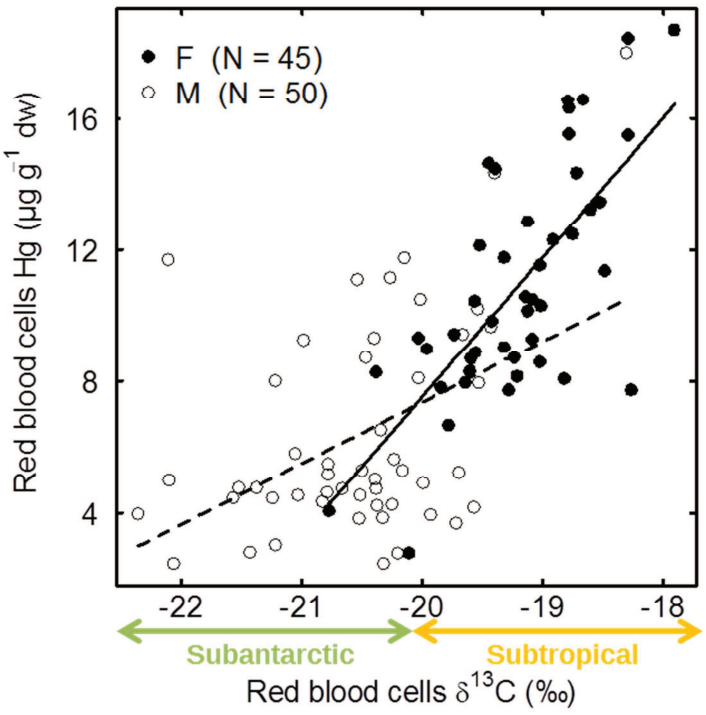

Fig. 22. *Relationships between blood Hg concentrations and foraging habitat (blood $\delta^{13}C$) in breeding wandering albatrosses from the Crozet Islands. The lines represent the best model fitting the data: Hg = $\delta^{13}C$ + sex + $\delta^{13}C$:sex (LM, explaining 53% of the total variation; solid and dashed lines for females and males, respectively; $\delta^{13}C$:sex represents the interaction between $\delta^{13}C$ and sex). Abbreviations: F, females; M, males. Modified from Fig.1 in **Paper 4**.*

General discussion. Overall, the concurrence of results at the community, population and individual levels indicates that, contrary to my prediction, Hg transfer to southern Indian Ocean seabirds increases from Antarctic to subantarctic and subtropical waters. This indirectly means that Hg bioavailability and/or food web transfer are different depending on the water masses. To the best of my knowledge, such a latitudinal gradient in Hg concentrations has never been shown in seabirds. A possible explanation of this unexpected pattern is the increasing **complexity of marine food webs** from high Antarctic to low subantarctic and subtropical latitudes. Antarctic food webs are rather simple, with upper predators relying directly or indirectly on a few key species, such as the Antarctic krill *Euphausia superba* and Antarctic silverfish *Pleuragramma antarcticum* (*e.g.*, Corsolini 2009). Conversely, predators in subantarctic and subtropical food webs feed on a large variety of prey species (*e.g.*, Cherel et al. 1999, Pinaud et al. 2005, Cherel et al. 2007). Food web structure influences Hg transfer (Cabana and Rasmussen 1994, Point et al. 2011), with an increasing number of trophic levels between predators and their prey resulting in higher Hg concentrations in the predators' tissues (Cabana et al. 1994). A greater biomagnification of Hg due to food web complexity, despite relatively low Me-Hg concentrations in the water (Cossa et al. 2011), could explain the high Hg burden in subtropical seabirds, especially at high trophic levels (*i.e.* subantarctic skua and wandering albatrosses, Fig. 21 and 22). Furthermore, it has recently been shown in the Arctic that Hg methylation could occur within a species of zooplankton (the copepod *Calanus hyperboreus*), probably mediated by its gut microbial communities (Pućko et al. 2014). While this recent discovery merits further investigation in other localities and zooplankton species, the **production of Me-Hg within the food web itself** could contribute to explain the uncoupling between Hg concentrations measured in predators (higher at low latitudes) and those measured in seawater (Me-Hg higher at high latitudes, Cossa et al. 2011). Alternatively, many other biotic and abiotic

factors may influence Hg bioavailability and food web transfer in different water masses, including **biological productivity**, **temperature**, or the distribution of other mineral and trace elements, but this still needs to be addressed. Finally, high Hg concentrations could be the result of the presence of particular geological sources in the subantarctic and subtropical region of the southern Indian Ocean. Clearly, these results call for in-depth investigations of seawater Hg speciation, distribution and food web dynamics in the Southern Ocean in different water masses, and in different seasons and years.

Conclusion. Hg transfer to seabirds gradually increases from Antarctic to subantarctic and subtropical water masses in the southern Indian Ocean. This hitherto undocumented latitudinal trend is not in accordance with previous knowledge on Me-Hg distribution in Southern Ocean waters, but likely derives from the concurrence of different factors, in particular food web complexity, which need to be further investigated.

4.1.2. Spatial variation in POPs transfer to seabirds

State of the art and hypothesis. According to global distillation (see Chapter 1), POPs burdens were found to be higher in biota from high than low latitudes in the Northern Hemisphere (*e.g.*, Muir et al. 1990, Kleivane et al. 1995, Wania and Mackay 1996, including seabirds: Steffen et al. 2006, Bourgeon et al. 2012, Sonne et al. 2013), especially when considering the most volatile compounds (*e.g.*, Simonich and Hites 1995). However, contrasting geographical patterns in the Northern Hemisphere have also been observed, probably in relation to the proximity to POPs sources, especially for heavily-chlorinated compounds (*e.g.*, Simonich and Hites 1995, Ter Schure et al. 2002, Bustnes et al. 2012). The Southern Hemisphere has different bio-geographical features than the Northern Hemisphere in that it is mainly covered by waters, far less populated and presents a relatively recent legacy of anthropogenic contamination (*e.g.*, Iwata et al. 1993, Connell et al. 1999). In

particular, the Southern Ocean is isolated from the main direct POPs sources (*e.g.*, Bargagli 2008, Corsolini 2009). In such a far-removed environment, POPs inputs derive mainly from atmospheric circulation, and global distillation mechanisms could be expected to contribute significantly to spatial variation in biota contamination, as shown for example in seabirds (Van den Brink 1997). *I therefore expected seabirds relying on Antarctic environments to show higher POPs burdens than those relying on subantarctic and subtropical environments*. In order to test this hypothesis I compared blood POPs residues in four populations of skua species from the different TAAF districts (Table A4 in the Appendix), and studied between-individual variation in adult wandering albatrosses from the Crozet Islands (**Paper 4**). Spatial differences have been evaluated by taking into account feeding ecology through stable isotopes and/or known dietary habits.

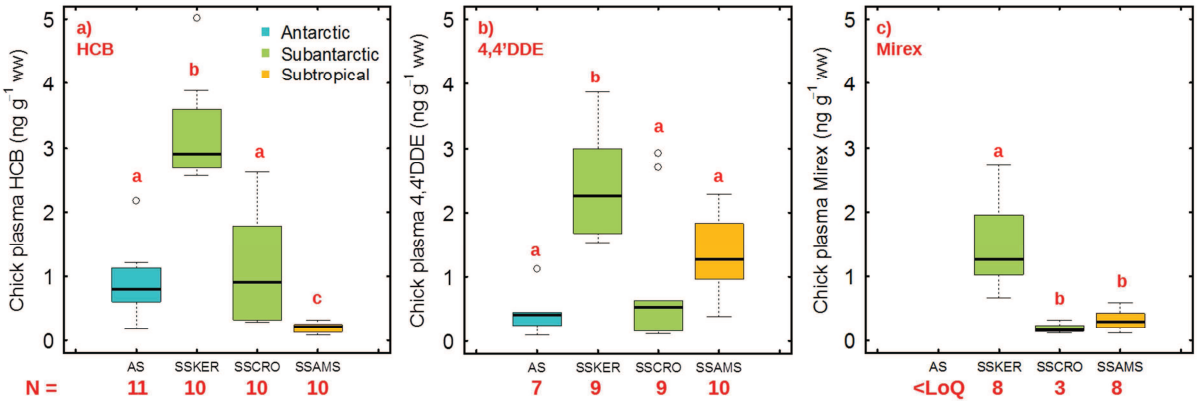

Fig. 23. *Box plots of blood **a)** HCB, **b)** 4,4'-DDE, and **c)** mirex concentrations (all on a logarithmic scale) in chicks of Antarctic skuas from Terre Adélie (AS) and three populations of subantarctic skuas from the Kerguelen (SSKER), Crozet (SSCRO) and Amsterdam (SSAMS) Islands. From a) to b) to c) the compounds have **decreasing volatility**. Skua populations sharing the same letters have similar blood OCPs concentrations (Wilcoxon comparisons, all $p < 0.05$). Unpublished data, **Table A4** in the **Appendix**.*

Results and discussion. The POPs pattern of skua chicks was largely dominated by OCPs (mainly HCB and 4,4'-DDE), while PCBs were usually under the limit of quantification (LoQ) (see Chapter 5 and Table A4 in the Appendix). Preliminary analyses highlighted important between-populations differences in OCPs concentrations, with for example blood HCB concentrations ranging from 0.2 ± 0.1 to 3.3 ± 0.8 ng g^{-1} ww in the least

and most contaminated populations (Amsterdam and Kerguelen subantarctic skua chicks, respectively, mean ± SD, Table A4). No clear latitudinal pattern was highlighted, irrespective of the relative volatility of the different compounds (HCB, 4,4'-DDE and mirex have decreasing volatility, *e.g.*, Wania and Mackay 1996). However, subantarctic skua chicks from the Kerguelen Islands showed consistently higher concentrations than the other skua populations (Fig. 23). This could derive from the fact that, at the study colony, subantarctic skua chicks are fed almost exclusively with blue petrels (Mougeot et al. 1998), which had among the highest POPs concentrations of all TAAF adult seabirds so far investigated, including penguins, petrels and albatrosses (for example blood HCB concentrations were 3.1 ± 2.8 *vs.* 2.2 ± 1.5 ng g^{-1} ww in adult blue petrels *vs.* wandering albatrosses, mean ± SD, Polartop unpublished data, see also Chapter 5). Therefore, global distillation does not seem to explain POPs contamination in skua chicks from the southern Indian Ocean. Instead, biomagnification mechanisms seem to outweigh the influence of spatial patterns of environmental POPs concentrations in these high-trophic-level seabirds (*e.g.*, Borgå et al. 2005, Steffen et al. 2006).

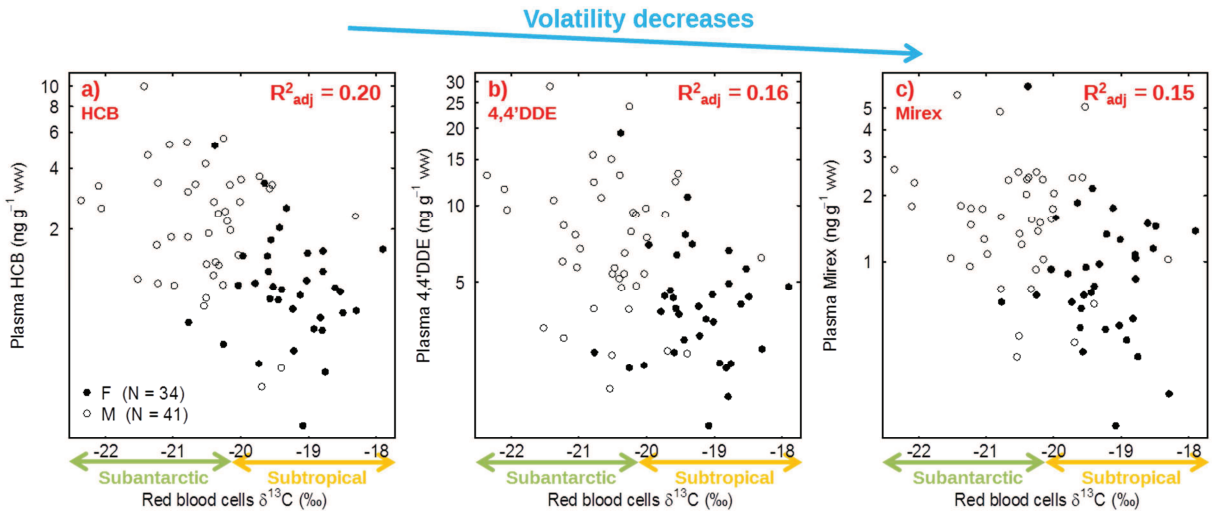

Fig. 24. *Relationships between blood* ***a)*** *HCB,* ***b)*** *4,4'-DDE, and* ***c)*** *mirex concentrations (all on a logarithmic scale) and foraging habitat (blood* $\delta^{13}C$*) in breeding wandering albatrosses from the Crozet Islands. From a) to b) to c) the compounds have* ***decreasing volatility****. The* R^2_{adj} *refer to LM applied on log-transformed OCPs concentrations: HCB:* $F_{1,73}=20$, $p < 0.001$; *4,4'-DDE:* $F_{1,73}=16$, $p < 0.001$; *mirex:* $F_{1,73} = 15$, $p < 0.001$. *In order to reduce multiple testing, multi-factorial analyses*

*were carried out only on the Σ_{11}OCPs, with the best model fitting the data being: Σ_{11}OCPs = δ^{13}C + sex (GLM, for more details see Paper 4). Abbreviations: F, females; M, males. Data from **Paper 4**.*

In the wandering albatross from the Crozet Islands, blood OCPs concentrations were significantly and negatively related to blood δ^{13}C values, indicating that individuals feeding in southern colder waters of the subantarctic zone had higher OCPs concentrations than those feeding in northern subantarctic and subtropical waters (section 3.5.2.2.). Moreover, the strongest relationship between blood OCPs and δ^{13}C values (and therefore latitude) was reported for the high volatile HCB (Fig. 24). Between-individual differences in OCPs concentrations of wandering albatrosses are consistent with the global distillation theory, as previously shown in other Antarctic seabirds (Van den Brink 1997, Bustnes et al. 2006, 2007), although such a clear latitudinal pattern has never been reported within a single population. Blood PCBs concentrations were overall not explained by blood δ^{13}C values (and therefore latitude), although the congener CB-180 was significantly and negatively related to blood δ^{13}C values (Fig. 25). This result is surprising, given the lower volatility and higher biomagnification potential of CB-180 compared to the other two congeners. However, the correlation explained only 8% of the total variation. Between-individual differences were extremely strong for blood PCBs concentrations, suggesting that intrinsic factors, in particular lipid dynamics or individual biotransformation capabilities, likely prevented the detection of potential spatial patterns.

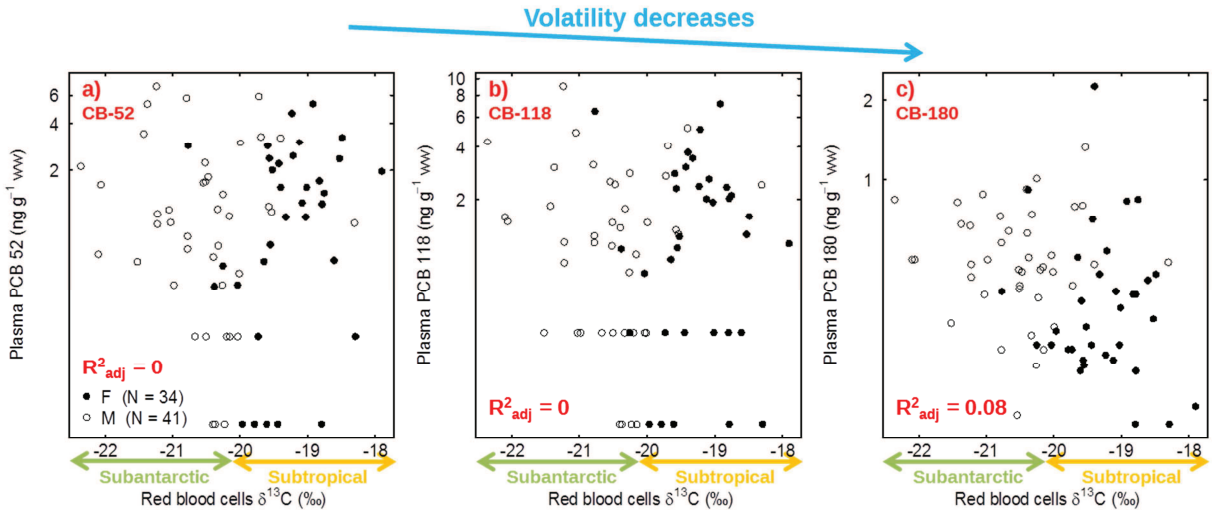

Fig. 25. *Relationships between blood **a)** CB-52, **b)** CB-118, and **c)** CB-180 concentrations (all on a logarithmic scale) and foraging habitat (blood $\delta^{13}C$) in breeding wandering albatrosses from the Crozet Islands. From a) to b) to c) the compounds have **decreasing volatility**. The R^2_{adj} refer to LMs applied on log-transformed PCBs concentrations: CB-52: $F_{1,73}$= 0, p = 0.93; CB-118: $F_{1,73}$= 0, p = 0.71; CB-180: $F_{1,73}$ = 7, p = 0.01. In order to reduce multiple testing, multi-factorial analyses were carried out only on the Σ_7PCBs, with the best model fitting the data being: Σ_7PCBs = Lipid content (GLM, for more details see Paper 4). Abbreviations: F, females; M, males. Data from **Paper 4**.*

Conclusions. POPs showed gradually increasing transfer to seabirds from northern warm to southern cold waters in the southern Indian Ocean, in accordance with the global distillation theory. Nevertheless, intrinsic physiological factors or differential biomagnification depending on compounds could prevent from inferring spatial patterns in POPs concentrations from high-trophic-level seabirds, especially for PCBs. In this regard, the use of one single species over a short time period could help to overcome the influence of several confounding factors.

4.2. Temporal trends of Hg transfer to seabirds: insights from penguin feathers

State of the art and hypothesis. Anthropogenic emissions of Hg have been higher than natural sources since the start of the industrial period (UNEP 2013). This legacy of emissions has led to an approximately three-fold increase in Hg circulating in the environment since pre-industrial times (Selin 2009). Global emissions are thought to have reached a maximum

during the 1970s, declined during the 1980s and early 1990s, and stayed relatively stable during the last decades, with decreases in Europe and North America being counterbalanced by increases in Asia (UNEP 2013). The Hg emission increase has been mirrored by an increase in Hg deposition since the 1890s, as shown by analysis of past Hg accumulation rates using lake sediments as geochemical archives both in the Northern and Southern Hemispheres (*e.g.*, Hermanns and Biester 2013). Thus the quantity of Hg available to living organisms has potentially increased since pre-industrial times. Since Hg is strongly bound to feather keratin, resisting vigorous chemical treatments (Appelquist et al. 1984), archives of feather samples from museum skins have served widely to investigate long-term temporal trends in bird Hg exposure, revealing contrasting patterns. Significant increases in feather Hg concentrations have been detected in seabirds from the Arctic environment (*e.g.*, Muir et al. 1999, Dietz et al. 2009) and from the North Atlantic (Thompson et al. 1992b, 1993, Monteiro and Furness 1997, Thompson et al. 1998b) and the Pacific (Vo et al. 2011) Oceans during the last century. Conversely, the few studies on feather Hg concentrations from seabirds of the Southern Ocean have shown different trends depending on species (Thompson et al. 1993, Becker et al. 2002, Scheifler et al. 2005), overall indicating a lack of widespread and pronounced temporal increase in Hg exposure during the 1900 century. During my doctoral thesis, temporal patterns of Hg concentrations were investigated in **penguins** through the analysis of feather samples from skins of museum specimens (Muséum National d'Histoire Naturelle, Paris, France) collected at Terre Adélie and the Crozet Islands in the **1950-1970s**. These samples were compared to feather Hg concentrations of contemporary (2007), free-living seabirds of the same species and from the same TAAF districts. Given the lack of consistent temporal trends in seabirds from the Southern Ocean (Thompson et al. 1993), *no hypothesis was formulated on potential temporal changes of feather Hg concentrations in TAAF penguins*.

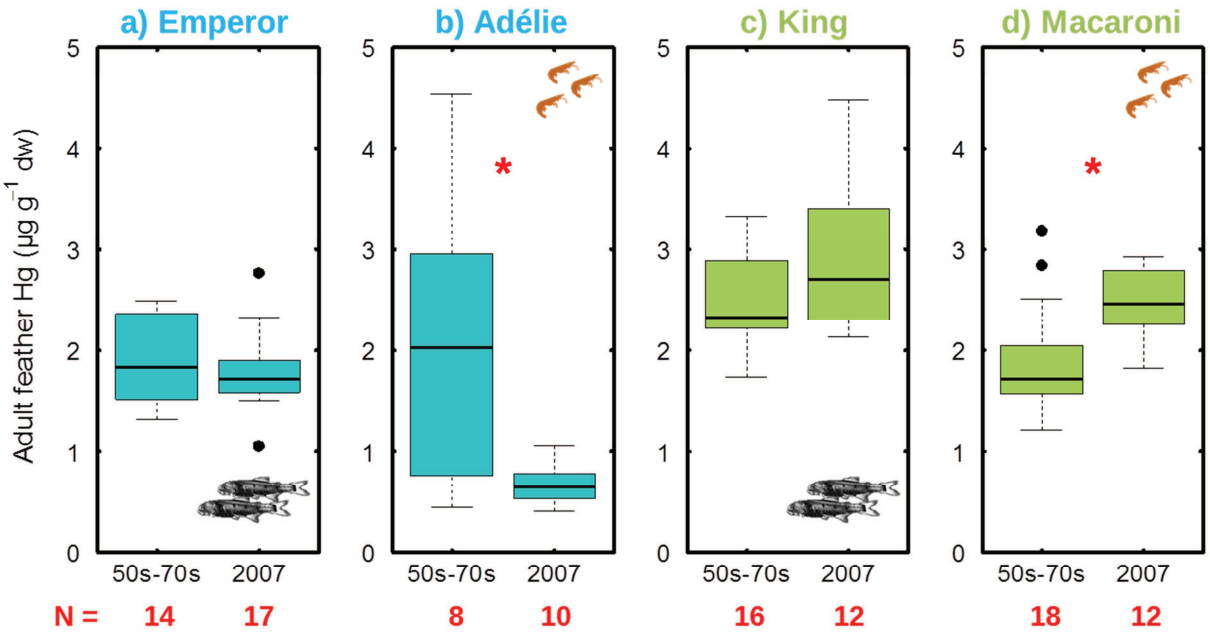

Fig. 26. *Box plots of feather Hg concentrations in Antarctic **a)** emperor and **b)** Adélie penguins from Terre Adélie, and subantarctic **c)** king and **d)** macaroni penguins from the Crozet Islands in the years 1950s-1970s and 2007. Since museum specimens of penguins were collected non-regularly through time, and given the small sample size, temporal trends were evaluated by group comparisons rather than classical time-series analyses. Therefore the asterisks indicate significant differences between the historic and contemporary groups (Wilcoxon comparisons, all p <0.05). Unpublished data, **Table A5** in the **Appendix**.*

Results and discussion. In accordance with previous observations in the Southern Ocean, temporal trends in feather Hg concentrations in penguins were different depending on species. The two mainly fish-eating species, the Antarctic emperor and subantarctic king penguins, showed no variation in feather Hg concentrations between the historical and contemporary samples (Fig. 26a,c). The mainly crustacean-eating Adélie penguin showed a significant decrease (-68%) in feather Hg concentrations between the two time periods, but the small sample size and the high between-individual variation of the historical sample call for caution in interpretation (Fig. 26b). Finally, the mainly crustacean-eating subantarctic species, the macaroni penguin, showed a significant increase (+32%) in feather Hg concentrations from the 1950s-1970s to present-day (Fig. 26d). These results can be interpreted in the light of penguin feeding habits, since stable isotopes were measured in the feathers of the same individuals (Jaeger and Cherel 2011). Feather $\delta^{15}N$ values indicated

slight species-specific dietary changes between the historical and contemporary samples. Namely, **Adélie and king penguins showed** no change in feather $\delta^{15}N$ values, indicating that they fed at **a similar trophic level in the 1950s-1970s and present-day**. Conversely, both **emperor and macaroni penguins** showed slightly, but significantly lower feather $\delta^{15}N$ values in 2007 than in the historical sample, indicating that they **recently fed less on fish and more on crustaceans** (Jaeger and Cherel 2011). The detected increase in feather Hg concentrations of macaroni penguins is therefore contradictory, and thus suggests that it was linked to increasing concentrations in the environment. Indeed, an increase in feather Hg concentrations have been recently detected in two other species of subantarctic seabirds, the black-browed *Thalassarche melanophrys* and grey-headed *Thalassarche chrysostoma* albatrosses from South Georgia, southern Atlantic Ocean (Becker et al. 2002), although potential temporal changes in feeding habits were not simultaneously assessed. Conversely, results on the king penguin are not in accordance with the only previous study on temporal trends of Hg exposure in this species (Scheifler et al. 2005). The authors indeed documented a significant decrease in king penguin feather Hg concentrations between the historical sample (based on the same museum specimens of the present doctoral work) and a sample collected in 2000-2001. This could indicate that from 2000-2001 to 2007 there has been an increase in feather Hg concentrations in king penguins from the Crozet Islands (from 2.0 ± 0.7 to 2.9 ± 0.7 µg g^{-1} dw, Scheifler et al. 2005 and Table A5 in the Appendix, respectively). This would be in accordance with the increasing trend observed in macaroni penguins, but feeding habits were not investigated in the 2000-2001 king penguin sample (Scheifler et al. 2005), preventing a pertinent interpretation of this potential temporal change in Hg exposure.

Conclusions. In contrast to results from Northern Hemisphere biota, there is a lack of a significant, consistent increase in Hg burdens in seabird species from the Southern Ocean. This likely indicates that measured concentrations in TAAF seabirds reflect **natural** Hg levels, in accordance with the low anthropogenic emissions that have characterised the Southern Hemisphere in the past centuries. Nevertheless, the increase detected in some subantarctic species may be indicative of recent changes of Hg emission patterns, linked to the development of Asian and Southern Hemisphere countries. Future monitoring is highly needed to confirm or not this trend, in particular in king and macaroni penguins, by using larger sample sizes and a larger time-window.

4.3. Summary

The investigation of spatio-temporal trends of environmental contamination through the use of seabirds necessitates understanding and controlling for the intrinsic and extrinsic factors that drive variation in exposure and bioaccumulation. By carefully selecting bioindicator species and by taking into account their feeding habits, my doctoral work has shown that:

- Hg transfer to seabirds **gradually increases** from Antarctic to subantarctic and subtropical latitudes in the southern Indian Ocean;
- in accordance with the global distillation theory, OCPs, and potentially PCBs, transfer to seabirds **gradually decreases** from Antarctic to subantarctic and subtropical latitudes in the southern Indian Ocean;
- blood POPs concentrations were likely more influenced by intrinsic factors than Hg, complicating the interpretation of spatio-temporal trends;
- Hg transfer to austral seabirds is overall not different today when compared to 50-70 years ago, but subantarctic species are possibly experiencing an increasing trend.

Chapter 5

Conclusions, Critical evaluation and Perspectives

Moulting wandering albatross chick on the Kerguelen Islands
Photo Thibaut Lacombe

5.1. Conclusions

5.1.1. Highlights of the doctoral work

This doctoral work provides important and new insights into the factors driving variation in seabird contaminant exposure and bioaccumulation, giving practical suggestions on the choice of bioindicator seabirds for monitoring of environmental contamination. By using accurately selected species as biomonitors, I have highlighted that important spatial and temporal trends in contaminant transfer to seabirds occurred in the southern Indian Ocean. More precisely, this thesis has shown that:

- **feeding ecology** is critical in driving variation in seabird contaminant concentrations in blood and feathers, at the community, species, sub-population and individual levels, irrespective of age class, age, sex and breeding status;
- **penguins** and **chicks of all species** can be used effectively as bioindicators, mainly because their moulting patterns and feeding habits are well known;
- **Hg transfer to seabirds gradually increases from Antarctic to subantarctic and subtropical waters** in the southern Indian Ocean, whereas **POPs transfer shows the opposite latitudinal trend**;
- **Hg transfer to seabirds of present-day is similar to that of 50-70 years ago**, with a potential increasing trend in subantarctic waters.

The doctoral work and dissertation have mainly focussed on Hg and legacy-POPs because of their potential toxicity, their capacity to bioaccumulate and biomagnify, and because their geochemical cycles and fate in marine organisms and food webs are relatively well-documented. Nevertheless, **the other poorly-studied trace elements and emerging-POPs** that have been measured during my doctoral work will be highlighted in upcoming scientific papers, such as **Paper 6** in the Appendix.

5.1.2. The main contaminants in the southern Indian Ocean and elsewhere

In order to identify contaminants that could be of concern and to put the results in a large context, an important aspect of my work has been to compare contaminant concentrations of TAAF seabirds to those of other (marine) bird species worldwide. Indeed, the first objective of my doctoral thesis was **descriptive** (see Chapter 1). Here, I present the main outcomes of this large comparative work, by focussing on Hg and organic compounds.

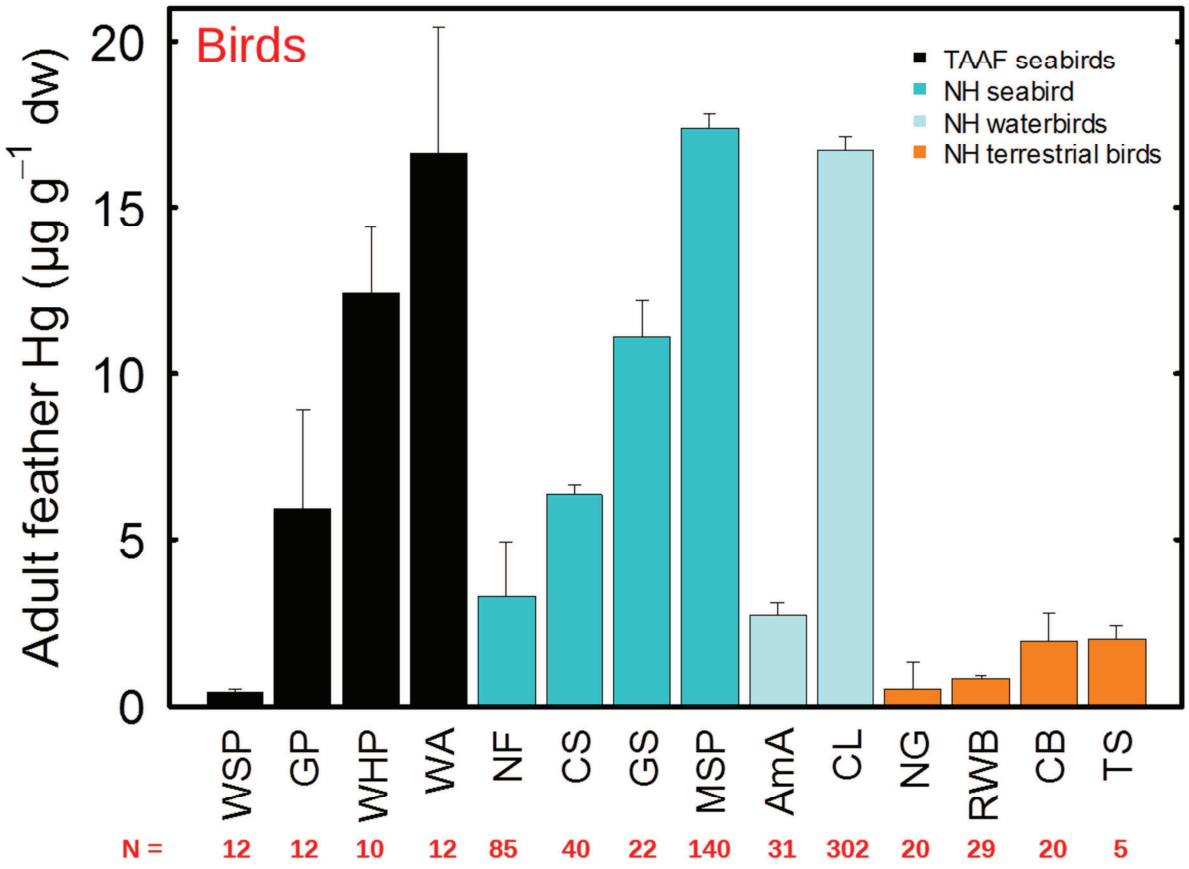

Fig. 27. *Bar plot of feather Hg concentrations in bird species from the TAAF (Kerguelen Islands) and the Northern Hemisphere (NH). Values are mean + SD for all species except AmA and CL, which are mean + SE, and NG and CB, which are median + absolute deviation. Abbreviations: WSP, Wilson's storm petrel; GP, gentoo penguin; WHP, white-headed petrel; WA, wandering albatross (data from Paper 3); NF, northern fulmar (Fulmarus glacialis, from Thompson et al. 1992a); CS, Cory's shearwater (Calonectris diomedea, from Monteiro et al. 1995); GS, great skua (from Thompson et al. 1998a); MSP, Madeiran storm petrel (Oceanodroma castro, from Monteiro et al. 1998); AmA, American avocet (Recurvirostra americana, from Eagles-Smith et al. 2009); CL, common loon (Gavia immer, from Evers et al. 2008); NG, northern goshawk (Accipiter gentilis, from Martinez et al. 2012); RWB, red-winged blackbird (Agelaius phoeniceus, from Tsipoura et al. 2008); CB, common buzzard (Buteo buteo, from Martínez et al. 2012); TS, tree swallow (Tachycineta bicolor, from Tsipoura et al. 2008).*

Mercury. Since most information on bird Hg concentrations is available on adult feathers, here I have chosen to focus on this tissue rather than on blood for comparisons. Results on feathers indicate that TAAF seabirds had a wide range of Hg concentrations, reflecting a large variation in exposure between species, as illustrated by the Kerguelen avian community (a factor of ~40 between the least and most exposed species). The pattern of feather Hg concentrations in adults of this community fits well with that of other subantarctic sites (Thompson et al. 1990, 1993, Anderson et al. 2009, Becker et al. 2002) but also to avian communities of oceanic islands in the Northern Hemisphere, such as the subtropical Azores Islands (North Atlantic Ocean, feather Hg concentrations ranging from approximately 1 to 20 $\mu g\ g^{-1}$; Monteiro et al. 1995, Monteiro et al. 1998, see Fig. 27). TAAF seabirds thus cover the entire range of feather Hg concentrations found worldwide in seabirds from remote areas. These comparisons also highlight that some seabird species from open sea regions are exposed to very high quantities of Hg, in particular when compared to terrestrial birds (Fig. 27). This is not surprising, since Hg biomagnification is exacerbated in aquatic environments (Fitzgerald et al. 2007, Ramade 2007, see Chapter 1). Notably, **the wandering albatross had among the highest feather Hg concentrations ever reported in birds**, as previously shown (*e.g.,* Anderson et al. 2009, Tavares et al. 2013), even when compared to top marine predators from industrialized countries, such as the great skua (Fig. 27). High Hg concentrations in wandering albatrosses are likely linked to slow moulting patterns and high exposure from the diet, which depends widely on large, deep water squids (Cherel and Weimerskirch 1999), which have high Me-Hg content (Bustamante et al. 2006, Bustamante et al. 2008).

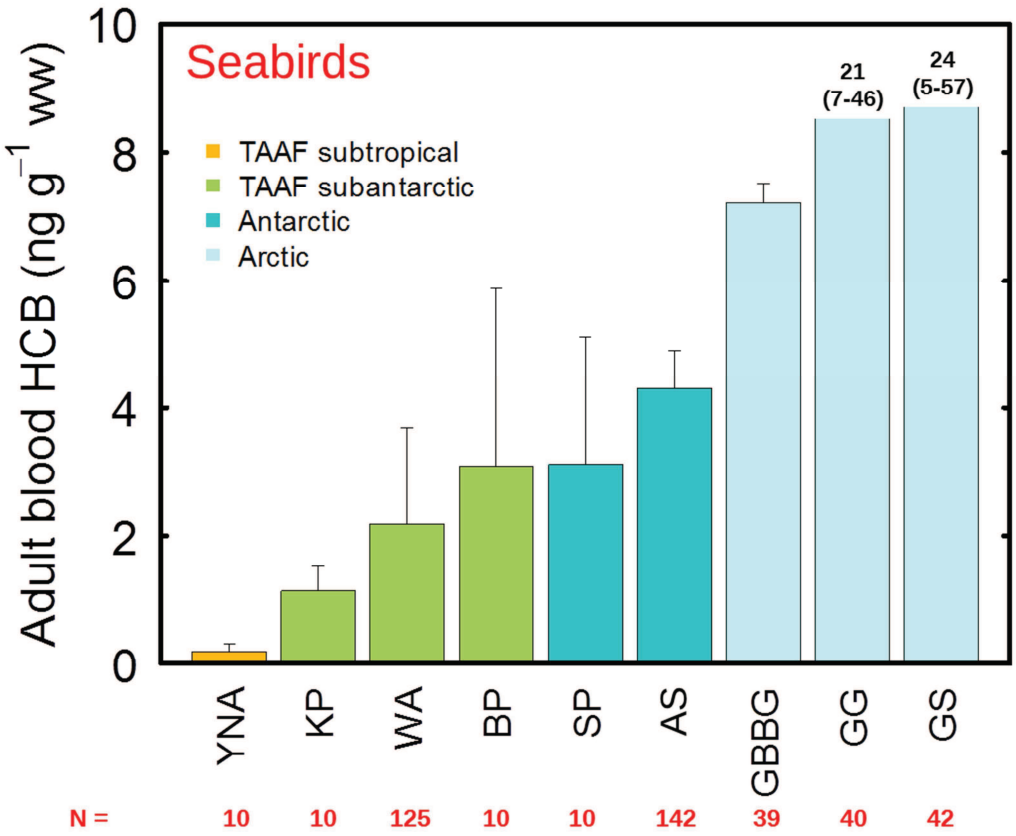

Fig. 28. *Bar plot of blood HCB concentrations in **adult** seabird species from the TAAF (YNA to SP), Antarctica (SPS) and the Arctic (GBBG to GS). Values are mean + SD for all species except AS and GBBG, which are mean + SE, and GG and GS, which are median and range in parenthesis. Abbreviations: YNA, Indian yellow-nosed albatross; KP, king penguin; WA, wandering albatross; BP, blue petrel; SP, snow petrel (data from Paper 3 and Polartop unpublished data on adults); AS, Antarctic skua (from Bustnes et al. 2006); GBBG, great black-backed gull (Larus marinus, from Bustnes et al. 2006); GG, glaucous gull (Larus hyperboreus, from Bustnes et al. 2006); GS, great skua (from Sonne et al. 2013).*

Persistent organic pollutants. Unlike Hg, during the doctoral work POPs concentrations have only been measured in plasma of chicks of different species (Table A6 in the Appendix) and of adult wandering albatrosses. Chicks of 15 TAAF species showed a wide range of POPs concentrations, with a factor of ~40 between the species with the lowest and highest Σ_{18}POPs concentrations (*i.e.* Adélie penguins and Kerguelen subantarctic skuas, respectively, Table A4 and A6 in the Appendix). Contamination by legacy-POPs in blood of seabirds has received little attention in the Southern Ocean, with most results being available on internal organs only (*e.g.*, Colabuono et al. 2012, Guruge et al. 2001a,b, Tanabe et al. 2004). Moreover, comparisons are made difficult by the use of different blood compartments

and/or different unities and by the fact that the sum of POPs, *e.g.*, ΣOCPs and ΣPCBs, are calculated from different compounds or congeners, respectively. Nevertheless, some information is available for **adult** seabirds from the Antarctic continent, which had comparable (penguin species, Van den Brink et al. 1998, Corsolini et al. 2007) or higher (Antarctic skua, Fig. 28, Bustnes et al. 2006, 2007) plasma POPs concentrations than adult wandering albatrosses. Furthermore, wandering albatrosses had lower POPs concentrations than seabirds from the Northern Hemisphere, including North Pacific albatrosses (Auman et al. 1997, Finkelstein et al. 2006, Harwani et al. 2011) and Arctic species, such as the glaucous gull *Larus hyperboreus*, which shows among the highest POPs concentrations in birds (*e.g.*, Bustnes et al. 2006, Fig. 28). Importantly, TAAF seabirds appeared to have a **smaller abundance of PCBs over OCPs**, in contrast to results in Northern Hemisphere seabirds (*e.g.*, Auman et al. 1997, Bustnes et al. 2006, Harwani et al. 2011). For example, in adult wandering albatrosses PCBs and OCPs accounted for 40% and 58% of the sum of POPs, respectively, and in chicks of 13 TAAF seabirds, PCBs were detected in only 21 out of 132 individuals (Table A6). Overall, lower concentrations of POPs and a lower abundance of PCBs in TAAF than Northern Hemisphere seabirds probably reflect the less industrialised character of the Southern Hemisphere. Nevertheless, some pesticides were particularly abundant in TAAF seabirds, for instance **HCB** and **mirex** (Fig. 29 for the wandering albatross). This is in accordance with recent studies documenting high concentrations of HCB and mirex in both the Antarctic atmosphere and biota (Van den Brink 1997, Goerke et al. 2004, Bustnes et al. 2007, Kallenborn et al. 2011), reflecting the mobility and use of these two compounds in the Southern Hemisphere (Connell et al. 2002, Bustnes et al. 2006). DDT derivatives also contributed strongly to the POPs pattern, as shown in the wandering albatross (Fig. 29), which is consistent with the continuous use of DDT in the Southern Hemisphere (Ritter et al. 1995, UNEP 2008).

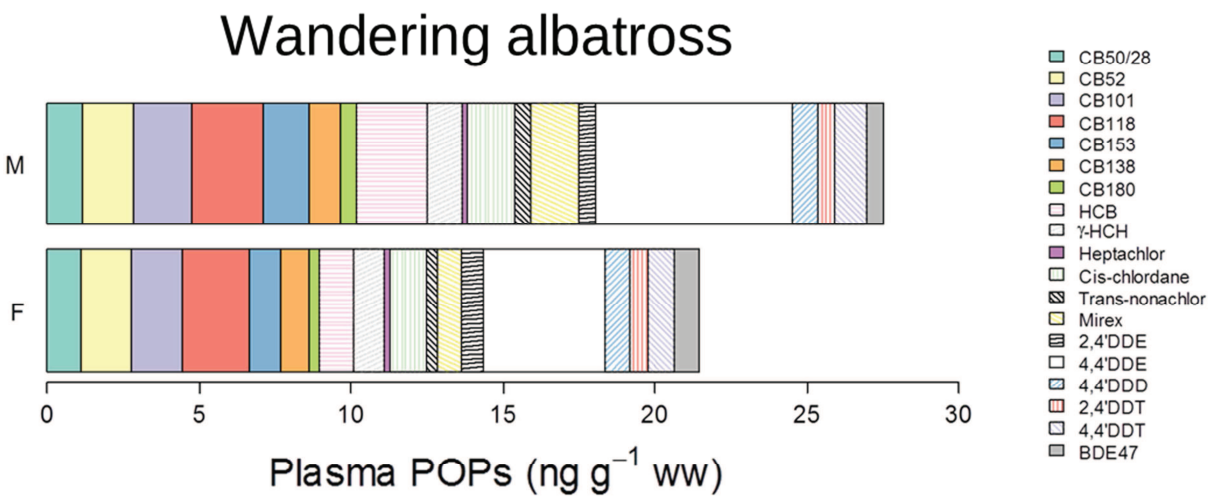

Fig. 29. *Stacked bar plot of POPs in plasma of male and female wandering albatrosses from the Crozet Islands (N = 128). Values correspond to median concentrations.*

5.1.3. Are the measured concentrations of concern?

Mercury. As illustrated in the previous section, Hg concentrations were very high in some TAAF seabirds when compared to other species worldwide, *but does this mean that birds could suffer adverse effects?* It is difficult to link observed Hg tissue concentrations to negative effects in natural bird populations because of a lack of experimental data and also because sensitivity varies between species (Burger and Gochfeld 2004). The most common used toxic threshold of feather Hg concentration in adult birds is 5 µg g^{-1} dw (*e.g.*, Eisler 1987, Burger and Gochfeld 1997, Evers et al. 2008). Within the Kerguelen avian community, 100% adult individuals of four species (wandering albatross, northern giant petrel, white-headed petrel *Pterodroma lessonii* and great-winged petrel *Pterodroma macroptera*) exceeded this threshold. Since seabirds appear to cope efficiently against high Hg exposure through detoxification processes, they are expected to have higher toxicity thresholds than terrestrial species (Scheuhammer 1987, Stewart et al. 1999). Indeed, in the common loon *Gavia immer*, an aquatic species with high Hg exposure, only concentrations > 35 µg g^{-1} dw in feathers were considered of high risk, with observed adverse effects on behaviour and reproduction (Champoux et al. 2006, Evers et al. 2008). No individual from the Kerguelen

avian community had feather Hg concentrations higher than this latter threshold (the maximum value was 32 µg g^{-1} dw in a northern giant petrel). Only experimental studies or robust investigations measuring tissue Hg concentrations together with health- or demographic parameters could assess the short- and long-term effects of Hg exposure (Evers et al. 2008). **Within the Polartop program**, evidence of deleterious Hg effects has been reported in TAAF seabirds at both the individual and population levels. In particular, the blood Hg concentrations measured during my doctoral work in wandering albatrosses (7.7 ± 3.6 µg g^{-1} dw, mean ± SD, N = 169) were associated to detrimental consequences on demographic parameters. Namely, blood Hg negatively impacted the breeding decision and the probability of hatching chicks, whereas adult survival was not affected (Goutte et al. 2014a). Furthermore, Hg concentrations were related to high oxidative damage level in the blood of the females (Costantini et al. 2014). In parallel, an in-depth study on adult subantarctic and Antarctic skuas from the Kerguelen Islands and Terre Adélie, respectively, have also shown that blood Hg concentrations (8.2 ± 0.2 and 2.2 ± 0.2 µg g^{-1} dw, mean ± SE, respectively) affected breeding success (Goutte et al. 2014b). Furthermore, blood Hg concentrations were related to changes in the secretion of a breeding-related hormone in young adult (≤ 23 years old) snow petrels from Terre Adélie (Tartu et al. 2014). *These results overall show that the level of Hg exposure measured in TAAF top predators is of concern*, since it may have subtle, long-term deleterious consequences on individuals and populations.

Persistent organic pollutants. Evaluating the toxicological significance of POPs concentrations in seabird tissues is complicated by several factors, including: 1) species-specific metabolic capacities, which lead to differential biotransformation of POPs within their tissues; 2) different toxicological mechanisms depending on the compounds, and possible synergistic and antagonistic effects between compounds ; and 3) the variable combination of POPs compounds being measured in each study (*e.g.*, Fisk et al. 2005, Letcher

et al. 2010, Muir and De Wit 2010). To facilitate risk assessment, the concept of toxic equivalency factors, or TEFs, has been developed (Van den Berg et al. 1998). This method is based on comparative toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD, the most toxic POP, and is applicable only for dioxins, furans and some PCBs (*dioxin-like* PCBs). During my doctoral work this method has not been used, since the Polartop program focuses on only one *dioxin-like* congener, the CB-118. Nevertheless, the large investigation on the wandering albatross has revealed that POPs burdens were negatively related to long-term demographic parameters (Goutte et al. 2014a). More precisely, blood POPs negatively affected the long-term probability of breeding and fledging chicks, while survival was not altered, similarly to Hg. POPs, like Hg, were also related to increased oxidative damage in plasma (Costantini et al. 2014). Since wandering albatrosses had lower POPs concentrations than seabirds from the Northern Hemisphere, such marked effects at both the individual and population levels are puzzling and provide further evidence that these anthropogenic compounds can exert detrimental effects at very low levels.

5.1.4. What are the best bioindicator species for biomonitoring?

Although the analysis of contaminant concentrations in blood can be highly informative, in particular to test relationships with physiological parameters (*e.g.*, hormones and biomarkers of oxidative stress), **feathers** are the **best tissue to sample for long-term monitoring** for three main reasons: 1) sampling feathers is easier and less invasive than sampling blood; 2) feathers are available in museums for historical comparisons; and 3) new archives can be easily realised and stored over the long-term.

As illustrated in Chapter 1, seabirds are good candidates for biomonitoring studies. Beyond the characteristics listed in Chapter 1, the most important criteria to select good bioindicator species for biomonitoring through **feathers** are (*e.g.*, Burger 1993):

- **first**, high contaminant concentrations, which enable an easy **quantification** and better **analytical precision**;
- **second**, low between- and within-individual variations in contaminant concentrations, which increase the **statistical power** necessary to describe spatio-temporal trends;
- **third**, constant feeding habits (a specialised diet), which make it possible to monitor a **well-defined food source**.

It is often not possible to find species fulfilling these criteria altogether. Hence, a good compromise between the different criteria must be found according to the contaminant and the monitoring goal. Importantly, feeding habits should be simultaneously assessed through stable isotopes or other techniques, in order to control for potential changes. The best candidate bioindicator species for Hg and POPs identified during the doctoral work are:

- **all species of penguins** because they have **(i) an annual complete and simultaneous moult of all feathers,** and **(ii) relatively constant feeding habits**, which make them representative of well-defined water masses (section 2.5.). In particular, because of their specialised diets, the **emperor**, **king** and **northern rockhopper penguins** appear to be ideal species for monitoring long-term temporal trends in Antarctic, subantarctic and subtropical waters, respectively. Conversely, penguins seem to be less suitable for POPs than Hg biomonitoring in the Southern Ocean, since they present low blood concentrations of several organic compounds (Table A6 for chicks and Polartop unpublished data for adults). Importantly, gentoo penguin populations that have a diversified diet are not suitable to be used for biomonotoring programs, because they display large between-individual variation in contaminant concentrations (section 3.5.2.1.);
- **chicks of all species of seabirds**, because they have a **(i) complete and simultaneous moult of all feathers before fledging** and a (**ii) well-known diet**, which is entirely

obtained from their parents from the vicinity of the breeding sites. Chicks are thus representative of a limited geographical region and of a well-defined time period (chick-rearing period). Nevertheless, two potential disadvantages could complicate the use of chicks as bioindicators of POPs: 1) the usually low detected concentrations and 2) a possible confounding influence of inherited burdens from the mother *via* the egg (Bourgeon et al. 2013);

- the **adult flying seabirds** that show relatively low between-individual variation in Hg concentrations (likely because of low between-individual variation in feeding habits), like the **light-mantled sooty albatross** (oceanic forager, mainly squid-eater), the **black-browed albatross** (inshore pelagic forager, mainly fish-eater) and the **Indian yellow-nosed albatross** (oceanic forager, mainly squid-eater). With regards to POPs, **blue petrels** (oceanic forager, mainly crustacean-eaters) and **snow petrels** (oceanic forager, mainly fish-eaters) seems to be good bioindicator species, given their relatively high blood POPs concentrations (Fig. 28). Finally, the **wandering albatross** has proved to be a good bioindicator of spatial variation in both Hg and POPs transfer to seabirds, but the high between-individual variation in contaminant concentrations implies that a large sample of individuals must be investigated for monitoring.

Ligth-mantled sooty albatross on the Kerguelen Islands (photo Alice Carravieri)

5.2. Critical evaluation and perspectives

5.2.1. What do blood and feather contaminant concentrations *really* mean?

In Chapter 2 I have made the assumption that contaminant concentrations in blood represent short-term exposure. Nevertheless, at any time, contaminant concentrations in blood result from two different contributions: 1) **recent dietary intake** (short-term exposure) and 2) **remobilisation** from internal tissues (long-term exposure). The relative proportion of these two fractions is **variable depending on the contaminant**. For example, some trace elements are stored within internal organs, with weak or no remobilisation (*e.g.*, inorganic Hg in liver, Cd in the kidney, Pb in bones, *e.g.*, Cuvin-Aralar and Furness 1991, Kim et al. 1998, Ikemoto et al. 2004), and their concentrations in blood mainly represent recent dietary intake. Moreover, the contribution of the short-term and long-term fractions into blood contaminant burdens may be **variable in time**, with dietary contribution outweighing the long-term fraction just after food assimilation, whereas the reversed situation may occur during **fasting** (Sandanger et al. 2003, Monteiro and Furness 2001). Despite this complex picture, contaminant toxicokinetics are understudied in seabirds, which may suffer low survival in laboratory conditions (Evers et al. 2008). In order to interpret correctly blood concentrations measured in TAAF species, I have tried to gather information on what is already known about Hg and POPs toxicokinetics in birds.

5.2.1.1. What are the biological half-lives of contaminants in blood?

Mercury. The few studies that have investigated the toxicokinetics of Hg in seabirds have shown that moult is a critical factor in determining Hg biological half-life in blood. For example, Cory's shearwater adults had blood biological half-lives in the range of **30-40 days** during moult, compared to **> 65 days** outside moult (Monteiro and Furness 2001). Hence, Hg concentrations in seabird tissues, *and blood*, might be relatively low towards the end and just

after moult, but increase once feathers are fully-grown (Bearhop et al. 2000c Monteiro and Furness 2001, Fournier et al. 2002). This implies that blood Hg concentrations before the onset of moult could largely represent the accumulated burden (Bearhop et al. 2000c). These results have important consequences for biomonitoring, indicating that **spatio-temporal comparisons should be done on individuals in the same moulting phase**. This could be difficult to realise in birds with protracted moult patterns, where the number of feathers growing at any time is unknown.

A better understanding of Hg toxicokinetics in blood would improve not only the use of blood as a monitoring unit, but also the interpretation of concentrations measured in feathers (Bearhop et al. 2000c).

Persistent organic pollutants. The whole-body biological half-lives of POPs have been reported to range from approximately **100 to 400 days** in birds and seabirds (Clark et al. 1987, Norstrom et al. 1986). Clearance from plasma depends on POPs physicochemical proprieties, which drive their partitioning within tissue lipid fractions. Namely, **low chlorinated PCBs** (*e.g.*, CB-28 and -52) and **chlordanes** (*e.g.*, γ-HCH) are **readily cleared** from plasma, since they are easily metabolised and poorly retained in tissue lipid fractions (Clark et al. 1987, Borlakoglu et al. 1990, Drouillard et al. 2001). On the other hand, **high chlorinated PCBs** (*e.g.*, CB-153 and -180), **mirex and 4,4'-DDE** are highly persistent, highly lipophilic, and **slowly metabolised** (*e.g.*, Clark et al. 1987, Borgå et al. 2001). Therefore, they partition among the various lipid pools relatively quickly to establish equilibrium concentrations between blood and tissue lipid fractions (Norstrom 2002). Consequently, **lipid dynamics** might affect the partitioning of POPs between adipose tissue and blood (Bustnes et al. 2010). Although fat accumulation or mobilisation lead to decreases or increases in the concentrations of circulating POPs, respectively, (Henriksen et al. 1998,

Van den Brink et al. 1998, Bustnes et al. 2010), these changes only marginally affected the evaluation of spatio-temporal trends (Bustnes et al. 2003). Lipid content in seabird blood varies between 0.3 and 0.8%, and within this range the repeatability of POPs values among individuals was high (Bustnes et al. 2001, 2007). This is consistent with results on the wandering albatross from the Crozet Islands, where plasma lipid content (0.6 ± 0.1%, mean ± SD, Table S3 in Supplementary Material of **Paper 4**) affected weakly blood POPs concentrations.

Better insights into the relationship between lipid dynamics and organic pollutants would however improve the ability to detect spatio-temporal trends from seabird blood.

5.2.1.2. Blood and feathers: a temporal uncoupling between contaminants and stable isotopes?

Blood. In order to use the stable isotope technique to explain contaminant exposure in seabirds, stable isotopes and contaminants should have the same temporal integration in the studied tissue. Unlike environmental contaminants, the turn-over of stable isotopes in bird tissues is relatively well known, including in different blood compartments (*e.g.*, Kelly 2000, Bearhop et al. 2002). **Stable isotopes in red blood cells represent feeding ecology over one to two months before sampling** (*e.g.*, Hobson and Clark 1992, 1993, Chapter 2), **which appears to be reasonably close to the known biological half-life of Hg in blood.** This is consistent with several studies showing correlations between stable isotopes and Hg in seabird blood (*e.g.*, Anderson et al. 2010, Bond and Diamond 2009b, this doctoral work). Nevertheless, given the long whole-body half-lives of POPs, there could be partial temporal uncoupling between POPs and stable isotope ratios in blood (Fisk et al. 2001a), in particular for slow-clearing, heavily chlorinated compounds. This implies carry-over effects of past

exposure (Bourgeon et al. 2012, Leat et al. 2013), especially in migrating species or species that have variable feeding habits.

Feathers. A significant proportion of the Hg body burden is excreted in the plumage during moult (*e.g.*, Braune and Gaskin 1987). A generally-admitted assumption (see Chapter 2) is thus that feather Hg concentrations represent the burden accumulated within the inter-moult period, while influence from dietary intake during moult is negligible (*e.g.*, Thompson et al. 1998a). **This implies that stable isotopes and Hg are temporally uncoupled in feathers of most adult birds**, as shown by the lack of correlations documented in several studies (*e.g.*, Thompson et al. 1998a, Ramos et al. 2009, Anderson et al. 2010). Some authors even argued that relationships between feather isotopic values and metals, including Hg, "are spurious and biologically uninformative" (Bond 2010). Nonetheless, as illustrated in this doctoral dissertation, this is not true for **chicks**, where stable isotopes and Hg are integrated over the chick-rearing period, and **penguins**, which have relatively constant isotopic niches. Furthermore, the doctoral work (unpublished data) has highlighted that, in some adult flying seabirds, **contaminant integration in feathers could, in certain instances, represent exposure during feather growth**, rather than the accumulated burden. This is exemplified by the case of subantarctic skuas at the Kerguelen Islands. During the breeding period, adults feed on seabird meat and present very high Hg concentrations in their blood (8.22 ± 0.24 µg g^{-1} dw, mean ± SE, Goutte et al. 2014b, comparable to that of the wandering albatross). Conversely, adult skuas have puzzling low Hg concentrations in feathers, half the level of chicks (section 3.3.1.). This contradictory finding depends on low Hg exposure from low trophic-level diet during the non-breeding period, with the **high Hg burden** accumulated during the breeding period being diluted in internal tissues and **gradually excreted in the sequentially growing feathers**. This interesting result merits further investigation, by

measuring stable isotopes and Hg on multiple feathers from the same individual, including, ideally, feathers that start growing on the breeding grounds.

Moreover, **moult patterns** are still poorly-known in all flying seabird species, especially for body feathers, which complicates the interpretation of feather contaminant concentrations.

5.2.1.3. Investigating contaminant remobilisation from energy stores in free-living seabirds during fasting

As illustrated above, the actual understanding of contaminant toxicokinetics in seabirds is limited, and in order to improve our capacity to interpret tissue concentrations, experimental studies should be realised on free-living individuals (Monteiro and Furness 2001). In this context, **penguins** seem to be ideal models to study environmental toxicokinetics in the wild, since they are easily accessible and stay on land for prolonged periods, in particular for **the beginning of the breeding cycle** (display, copulations and incubation) and **moult**, during which they fast (Williams 1995). This offers a unique opportunity to investigate the kinetics of remobilisation of environmental compounds from internal tissues **in natural conditions without the confounding effect of dietary intake**, which interferes with both lipid-content and contaminant concentrations (Bustnes et al. 2012). Since there is differential utilisation of lipid and protein stores throughout the fasting period (Cherel et al. 1994a), it could be possible to investigate contaminant changes not only in relation to lipids but also to proteins, which might influence the dynamics of some emerging-POPs (*e.g.*, PFOS, Verreault et al. 2007). Importantly, the simultaneous sampling of blood and feathers during the moult would enable clarifying the poorly-known intrinsic deposition of POPs into feathers (García-Fernández et al. 2013), which has never been studied in seabirds, to the best of my knowledge.

5.2.2. Can we better identify the source of contaminant exposure in seabirds?

As shown in my doctoral work, the stable isotope technique can be very powerful in depicting the relationship between contaminants and feeding ecology in seabirds. Nevertheless, isotopic values represent a crude estimation of feeding habitat. For example, in the southern Indian Ocean it is not possible to identify the longitudinal variation in feeding habitats from $\delta^{13}C$ values (Jaeger et al. 2010a). Furthermore, the use of $\delta^{13}C$ values to depict feeding habitats largely relies on the existence, and knowledge, of $\delta^{13}C$ gradients in the environment. In order to identify feeding habitats more finely, techniques of movement investigation could be applied, such as **seabird tracking** with Global Position Systems (GPS) or Global Location Sensing (GLS). This could be particularly useful in those species that perform wide-ranging migration (Bourgeon et al. 2013, Leat et al. 2013), such as the Antarctic skua, which reaches the Northern Hemisphere, where it could be exposed to different mixtures and quantities of contaminants.

$\delta^{15}N$ values give a good estimation of seabird trophic level in the southern Indian Ocean (Cherel et al. 2010) and are a powerful means to explain contamination patterns within communities and populations (Chapter 3). Nevertheless, some studies have shown that the presence of a particular prey type in the diet may be more important than trophic level *per se* in explaining contaminant burdens in some seabirds (Thompson et al. 1998b, Arcos et al. 2002). **Measuring contaminant concentrations in seabird prey could thus be necessary in some cases**. Unexpected patterns of contamination based on trophic level alone have been highlighted during my doctoral work. For instance, small zooplankton-eating petrels from the Kerguelen Islands (blue petrels and Antarctic prions) displayed surprisingly high concentrations of Cd and POPs in their blood and internal tissues (Polartop unpublished data, Table A7, **Paper 6**). While high Cd exposure could result from feeding on the pelagic amphipod *Themisto gaudichaudii*, which has high Cd content in Kerguelen waters (Bocher et

al. 2003), their high POPs burden is more difficult to explain without complementary studies on the contamination of their prey. Furthermore, given the recent finding on Hg methylation within a zooplankton species (*i.e. Calanus hyperboreus*, Pućko et al. 2014), and the high Hg transfer to seabirds in subtropical waters of the southern Indian Ocean, it would be interesting to study the distribution of organic and inorganic Hg species in plankton species of this region.

5.2.3. Are contaminants threatening the immune system of TAAF seabirds?

As illustrated in Chapter 1, environmental contaminants can cause **immunosuppression**, increasing susceptibility to disease (De Swart et al. 1996, Grasman 2002). For instance, POPs have been shown to increase the abundance of intestinal parasitic nematodes, or to alter the immune response in Arctic seabirds (*e.g.*, Sagerup et al. 2000, Letcher et al. 2010). Several biomarkers of the immune function can be non-destructively measured in blood (*e.g.*, haematocrit, lymphocytes counts, and immunoglobulins). For example, by using non-destructive biomarkers, Finkelstein et al. (2007) have shown that both Hg and POPs altered the immune function of a Northern Hemisphere albatross. In recent decades, some populations of TAAF seabirds have been affected by disease outbreaks (Weimerskirch 2004). In particular, some colonies of the Indian yellow-nosed albatross breeding on the subtropical Amsterdam Island seem to be affected by **avian cholera** (Rolland et al. 2009). The disease, caused by a bacterium (*Pasteurella multicoda*), reduces markedly chick survival and can induce mortality also on adults, with the studied population decreasing dramatically since the 1980s (Weimerskirch 2004). The sympatric sooty albatross *Phoebetria fusca*, which is classified as an endangered species like the Indian yellow nosed albatross (IUCN Red List 2011), seems also to be affected (Weimerskirch 2004). Studying the relationship between contaminants and the immune function in these species could contribute to understand the

spreading of the disease. This would be particularly relevant with regards to Hg, since the blood concentrations of both the Indian yellow-nosed and the sooty albatrosses are particularly high (10.3 ± 2.6 and 12.0 ± 3.3 μg g^{-1} dw, respectively, Polartop unpublished data).

5.2.4. Monitoring future trends of contamination in the southern Indian Ocean

The remote location of all TAAF districts makes them ideal study sites to monitor wide-range trends of environmental contaminants without the confounding effect of point sources. **Continuous monitoring of these remote southern environments is particularly relevant in the actual context of changing anthropogenic pollution patterns and global warming**. For instance, Hg emissions from Asian and Southern Hemisphere countries have greatly increased in the last decades, with global emissions staying stable despite recent international restrictions (UNEP 2013). Transfer of Hg emission to unavailable pools (*e.g.*, the deep ocean) happens at the scale of centuries, with the ocean being not in equilibrium with the current atmospheric Hg pool. Thus Hg deposition and Hg in surface reservoirs will likely increase in the next 50 years (Amos et al. 2013, Driscoll et al. 2013). Furthermore, global warming is believed to favour the methylation rate of Hg in the ocean, in particular through reduction in the levels of dissolved oxygen (Cossa 2013).

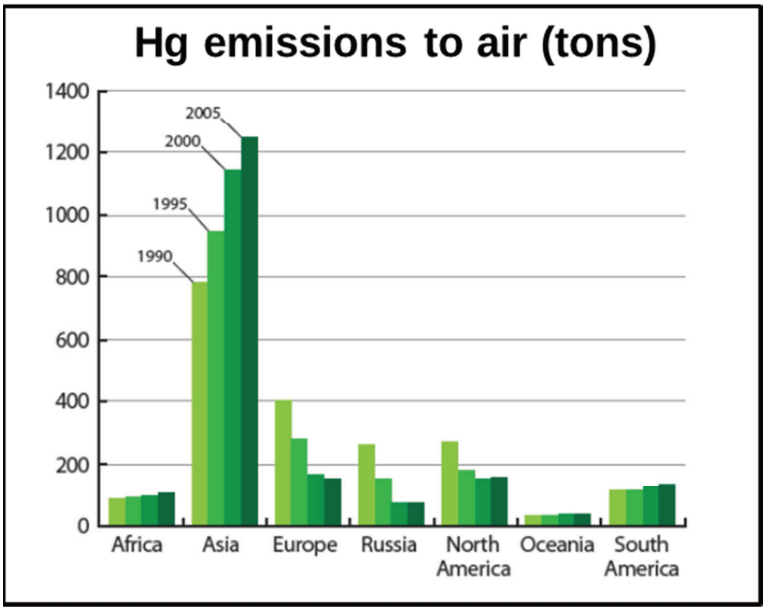

Fig. 30. *Estimates of annual anthropogenic Hg emissions from different continents/regions, 1990-2005 (from UNEP 2013).*

Continuous monitoring of legacy-POPs is also a priority, given their persistence, their current use in several Southern Hemisphere countries (UNEP 2008), and the deleterious effects that they elicit in biota. Nevertheless, there is an urgent need to evaluate also the distribution and trophic transfer of other contaminants, such as **emerging-POPs**, in particular PFOS, which may bioaccumulate in high quantities in predators and exert toxic effects (Verreault et al. 2007). There is increasing concern also with respect to **microplastic** (< 5 mm length) in the ocean, which have been detected in large quantities even in the Antarctic environment (Ivar do Sul et al. 2011). These materials are ingested intentionally and non-intentionally by many seabirds, especially albatrosses and petrels, causing physical damage and being the vector of several organic compounds (Moore 2008, Tanaka et al. 2013, Ivar do Sul and Costa 2014). To the best of my knowledge, plastic ingestion has never been studied in TAAF seabirds, but could potentially elucidate part of their exposure to POPs.

The best bioindicator species identified in the present work could be used effectively to monitor future **temporal trends in environmental contamination** in the Southern Ocean. Recently-established monitoring programs in the TAAF enable **sampling feathers annually**

from different species, including the blue petrel, king penguin, black-browed albatross (subantarctic), emperor penguin, snow petrel (Antarctic), Indian yellow-nosed albatross and northern rockhopper penguin (subtropical). Therefore, future research efforts should be focussed on contaminant evaluation in **feathers, including notably POPs determination** (Jaspers et al. 2007, García-Fernández et al. 2013). Finally, measuring POPs in feathers of museum specimens would give invaluable insights into the poorly-known temporal trends of organic contamination in the Southern Ocean.

Indian yellow-nosed albatross on Amsterdam Island (photo Jérémie Demay)

Black-browed albatross on the Kerguelen Islands (photo Thibaut Lacombe)

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Appendix

Pawel Kuczynski, 2008

Materials and Methods – Summary

The preparation of blood and feather samples and their analysis for environmental contaminant concentrations followed classical techniques that are detailed in the scientific papers (see other appendices), together with statistical analyses. Here I summarise the most important analytical steps.

Sample preparation

In the field, 6-10 body feathers were sampled randomly from the lower back and then stored in sealed plastic bags. Blood was sampled from the tarsal or the brachial vein with heparinized syringes (2-6 ml depending on the species body mass). Red blood cells and plasma were separated by centrifugation within 3 hours and stored at -20°C until analyses.

Feathers were vigorously washed with organic solvents (chloroform/methanol, 2/1; v/v), oven-dried for 48 hours at 50°C and cut into small fragments (1-2 mm length) for homogenisation. Whenever possible, Hg determination was realised on individual feathers (0.5-3 mg subsamples) from each individual, whereas multiple feathers were pooled to have enough material (20-200 mg) for the analysis of the other trace elements.

Red blood cells were freeze-dried and ground to powder before analyses of **stable isotopes** (~0.3 mg), **Hg** (2-20 mg subsamples) and other **trace elements** (20-200 mg subsample), in this order. **Plasma** was used for **POPs** determination (100-300 µl aliquots).

Sample analyses

Stable isotopes, Hg and other trace elements were measured at the laboratory **LIENSs**, La Rochelle, France. POPs were measured at the laboratory **EPOC-LPTC**, Bordeaux, France.

Stable isotopes. The relative abundance of stable isotopes was determined in red blood cells and feathers subsamples with a **continuous flow mass spectrometer coupled to an elemental analyser**. Results are in the usual δ notation relative to Vienna PeeDee

Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors < 0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. Results are given in ‰ as means ± SD.

Mercury. Feathers and red blood cells were analysed for Hg in an **Advanced Mercury Analyzer spectrophotometer (Altec AMA 254)**. Measurement quality was assessed by the certified reference material Tort-2 Lobster Hepatopancreas (NRC, Canada). All analyses were repeated 2–3 times until having a relative SD < 10%. Blanks were analysed at the beginning of each set of samples and the limit of detection (LoD) was 0.005 μg g^{-1} dw. Results are given in μg g^{-1} dw as means ± SD.

Trace elements. As, Cr, Cu, Fe, Mn, Ni, Se and Zn were analysed by **Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)** and Ag, Cd, Co, Pb and V by **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**. Feathers and red blood cells were mineralised in a microwave before analysis with a mixture of nitric and chlorhydric acids. Measurement quality was assessed by the certified reference materials Tort-2 Lobster Hepatopancreas and Dolt-4 Dogfish Liver (both NRC, Canada), which were treated and analysed in the same way as the samples. For each set of analyses, blanks were included in each analytical batch. The LoD (μg g^{-1} dw) were 0.015 (Cd), 0.017 (Ag), 0.02 (Cr, Co, Pb), 0.03 (Ni), 0.08 (Mn), 0.1 (Cu, Se), 0.2 (As), 0.3 (V) and 3.3 (Fe and Zn). Results are given in μg g^{-1} dw as means ± SD.

Persistent organic pollutants. Targeted compounds included 11 organochlorine pesticides (OCPs: cis-chlordane, trans-nonachlor, HCB, γ-HCH, heptachlor, mirex, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT), 7 polychlorinated biphenyls (PCBs: CB-28, -52, -101, -118, -138, -153 and -180) and 12 polybrominated diphenyl ethers (BDE-17, -28, -49, -71, -47, -66, -100, -99, -154, -153, -183 and -209). Internal standards were used for quantification (CB-30, -103, -155, -198 for PCBs, p,p'-DDT-d8 for OCPs and F-BDE-47,

BDE-181, -209 for PBDEs). After extraction (pentane/dichloromethane, 90/10; v/v), purification on an acid silica gel column and elution (pentane/dichloromethane, 90/10; v/v), extracts were re-concentrated and added to isooctane as solvent keeper. Final extracts were analysed by **gas chromatography (GC) coupled with a ^{63}Ni electron capture detector (ECD)**. Conversely, PBDEs were analysed by **gas chromatography coupled with mass spectrometry operated in negative chemical ionisation (GC-NCI-MS),** except BDE-47 in wandering albatross plasma, which was measured by GC-ECD. CB-28 coeluted with CB-50 in all samples, and is reported as CB-28/50. POPs concentrations were blank corrected and the LoD was set at two times the mean blank value; for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of 3. Quality control consisted in the analysis of procedural blanks (clean and empty glass tubes treated like a sample, one run for 8 samples). Chicken plasma aliquots spiked with known concentrations of POPs were analysed in parallel to the samples to assess recovery rates. Lipid content was determined in wandering albatross plasma (10 µL aliquots) by the sulfo-phospho-vanillin method for colorimetric determination and by gravimetry for Antarctic prions internal tissues. Results are given in both absolute concentrations in ng g^{-1} ww and relative to the plasma lipid weight (lw) as means ± SD, unless otherwise indicated. POPs LoD and LoQ are repoterd in the scientific papers, since LoD and LoQ changed depending on the study.

Tables

Table A1. Summary of sampled individuals for the Polartop program (season 2011-2012) and previous CEBC programs. **The total number of samples included in the doctoral work is N = 1472 (612 chicks and 860 adults)**. Not included in my doctoral work are: blood and feather data from adult birds sampled in 2011-2012 (indicated by *) and feather data on the wandering albatross (indicated by **). These data will be included in upcoming Polartop papers (C. Cipro, P. Bustamante, and A. Carravieri).

Species	District[1] Year of sampling	Chicks n	Chicks Blood	Chicks Feather	Adults n	Adults Blood	Adults Feather
Spheniscidae							
Emperor penguin	TA, 2007	10	-	SI, Hg*	17	-	SI, Hg
(*Aptenodytes forsteri*)	TA, Historical	-	-	-	14	-	SI, Hg
	TA, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	-	-	-
King penguin	KER, 2007	12	-	SI, Hg	12	-	SI, Hg
(*Aptenodytes patagonicus*)	CRO, 2007	12	-	SI, Hg	12	-	SI, Hg
	CRO, 2005	-	-	-	7	-	SI, Hg
	CRO, Historical	-	-	-	17	-	SI, Hg
	CRO, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	21*	SI, Hg, TE, POPs	SI, Hg
Gentoo penguin	KER, Estacade 2007	12	-	SI, Hg	12	-	SI, Hg
(*Pygoscelis papua*)	KER, Penn Island 2007	-	-	-	12	-	SI, Hg
	CRO, 2007	12	-	SI, Hg	12	-	SI, Hg
	CRO, Historical	-	-	-	12	-	SI, Hg
	CRO, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	21*	SI, Hg, TE, POPs	SI, Hg
Adélie penguin	TA, 2007	10	-	SI, Hg	10	-	SI, Hg
(*Pygoscelis adeliae*)	TA, Historical	-	-	-	8	-	SI, Hg
	TA, 2011-12	14	SI, Hg, TE, POPs	SI, Hg (10)	26*	SI, Hg, TE, POPs	SI, Hg
Macaroni penguin	KER, 2007	12	-	SI, Hg	12	-	SI, Hg
(*Eudyptes chrysolophus*)	CRO, 2007	12	-	SI, Hg	12	-	SI, Hg
	CRO, Historical	-	-	-	18	-	SI, Hg
	CRO, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	20*	SI, Hg, TE, POPs	SI, Hg
Southern rockhopper penguin	KER, 2007	12	-	SI, Hg	12	-	SI, Hg
(*Eudyptes chrysocome filholi*)	CRO, 2007	12	-	SI, Hg	12	-	SI, Hg
	CRO, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	20*	SI, Hg, TE, POPs	SI, Hg
Northern rockhopper penguin	AMS, 2007	15	-	SI, Hg	12	-	SI, Hg
(*Eudyptes chrysocome moseleyi*)	AMS, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	20*	SI, Hg, TE, POPs	SI, Hg
Diomedeidae							
Wandering albatross	KER, 2005, 06	15	-	SI, Hg	12	-	SI, Hg
(*Diomedea exulans*)	CRO, 2008	-	-	-	180	SI, Hg, TE, POPs	SI, Hg**
Amsterdam albatross	AMS, 2011-12	11	SI, Hg, TE, POPs	SI, Hg	-	-	-
(*Diomedea Amsterdamensis)*							
Black-browed albatross	KER, 2005, 06	18	-	SI, Hg	33	-	SI, Hg
(*Thalassarche melanophrys*)							
Indian yellow-nosed albatross	AMS, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	20*	SI, Hg, TE, POPs	SI, Hg
(*Thalassarche carteri*)							
Light-mantled sooty albatross	KER, 2005, 07	15	-	SI, Hg	16	-	SI, Hg
(*Phoebetria palpebrata*)							
Sooty albatross	AMS, 2011-12	-	-	-	11	SI, Hg, TE, POPs	SI, Hg
(*Phoebetria fusca*)							
Procellariidae							
Northern giant petrel	KER, 2005, 08	12	-	SI, Hg	18	-	SI, Hg
(*Macronectes halli*)							
Grey petrel	KER, 2005	16	-	SI, Hg	16	-	SI, Hg
(*Procellaria cinerea*)							
White-chinned petrel	KER, 2005	14	-	SI, Hg	14	-	SI, Hg
(*Procellaria aequinoctialis*)	CRO, 2007	10	-	SI, Hg	-	-	-
	CRO, 2011-12	11	SI, Hg, TE, POPs	SI, Hg	10*	SI, Hg, TE, POPs	SI, Hg

Species	District[1] Year of sampling	Chicks n	Chicks Blood	Chicks Feather	Adults n	Adults Blood	Adults Feather
Great-winged petrel (*Pterodroma macroptera*)	KER, 2005	10	-	SI, Hg	14	-	SI, Hg
White-headed petrel (*Pterodroma lessonii*)	KER, 2003	10	-	SI, Hg	14	-	SI, Hg
Soft-plumaged petrel (*Pterodroma mollis*)	KER, 2010, 11	-	-	-	19	-	SI, Hg
Kerguelen petrel (*Aphrodroma brevirostris*)	KER, 2007, 08, 09, 10	18	-	SI, Hg	24	-	SI, Hg
Snow petrel (*Pagodroma nivea*)	KER, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	24*	SI, Hg, TE, POPs	SI, Hg
Blue petrel	KER, 2003	13	-	SI, Hg	25	-	SI, Hg
(*Halobaena caerulea*)	KER, 2011-12	11	SI, Hg, TE, POPs	SI, Hg	31*	SI, Hg, TE, POPs	SI, Hg
Antarctic prion	KER, 2008	10	-	SI, Hg	10	-	SI, Hg
(*Pachyptila desolata*)	KER, 2012	-	-	-	10	SI, Hg, TE	SI, Hg, TE
Thin-billed prion	KER, 2003	9	-	SI, Hg	20	-	SI, Hg
(*Pachyptila belcheri*)	KER, 2011-12	12	SI, Hg, TE, POPs	SI, Hg	24*	SI, Hg, TE, POPs	SI, Hg
Hydrobatidae							
Wilson's storm petrel (*Oceanites oceanicus*)	KER, 2005	-	-	-	12	-	SI, Hg
Black-bellied storm petrel (*Fregetta tropica*)	KER, 2005, 10	-	-	-	10	-	SI, Hg
Grey-backed storm petrel (*Garrodia nereis*)	KER, 2006	-	-	-	23	-	SI, Hg
Pelecanoididae							
Common diving petrel (*Pelecanoides urinatrix*)	KER, 2003	17	-	SI, Hg	29	-	SI, Hg
South Georgian diving petrel (*Pelecanoides georgicus*)	KER, 2009, 10, 12	16	-	SI, Hg	24	-	SI, Hg
Phalacrocoracidae							
Kerguelen shag (*Phalacrocorax verrucosus*)	KER, 2005, 06	10	-	SI, Hg	30	-	SI, Hg
Stercorariidae							
Subantarctic skua	KER, 2005, 09, 10	22	-	SI, Hg	26	-	SI, Hg
(*Catharacta lönnbergi*)	KER, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	55*	SI, Hg, TE, POPs	SI, Hg, TE, POPs
	CRO, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	-	-	-
	AMS, 2011-12	10	SI, Hg, TE, POPs	SI, Hg	12*	SI, Hg, TE, POPs	SI, Hg, TE, POPs
Antarctic skua (*Catharacta maccormicki)*	TA, 2010-11	11	SI, Hg, TE, POPs	SI, Hg	54*	SI, Hg, TE, POPs	SI, Hg, TE, POPs
Laridae							
Kelp gull (*Larus dominicanus*)	KER, 2010, 11	7	-	SI, Hg	5	-	SI, Hg
Chionididae							
Lesser sheathbill (*Chionis minor*)	KER, 2009, 10	-	-	-	26	-	SI, Hg
Anatidae							
Kerguelen Pintail (*Anas eatoni eatoni*)	KER, 2010	-	-	-	16	-	SI, Hg

[1] TA, Terre Adélie, CRO, Crozet Islands, KER, Kerguelen Islands, AMS, Amsterdam Island.

Table A2. Feather Hg concentrations and stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values in penguin species breeding at different TAAF districts. For district abbreviations see Table A1.

						Hg (µg g^{-1} dw)			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Species	**Abb.**	**District**	**Water mass**	**Year**	**N**	**Mean ± SD**	**Range**	**Median**	**Mean ± SD**	**Mean ± SD**
Fish-eating										
Emperor	EP	TA	Antarctic	2007	17	1.77 ± 0.37	1.05-2.76	1.71	-23.1 ± 0.3	12.2 ± 0.3
King	KP	CRO	Subantarctic	2007	12	2.89 ± 0.73	2.13-4.47	2.71	-20.8 ± 0.6	11.3 ± 0.5
Crustacean-eating										
Adélie	AP	TA	Antarctic	2007	10	0.66 ± 0.20	0.41-1.06	0.65	-23.4 ± 0.4	10.7 ± 0.6
Macaroni	MP	CRO	Subantarctic	2007	12	2.48 ± 0.35	1.82-2.92	2.47	-21.1 ± 0.3	9.7 ± 0.3
Southern rockhopper	SRP	CRO	Subantarctic	2007	12	1.79 ± 0.37	1.20-2.51	1.81	-21.1 ± 0.2	8.9 ± 0.4
Northern rockhopper	NRP	AMS	Subtropical	2007	12	1.82 ± 0.30	1.42-2.34	1.80	-17.9 ± 0.3	11.3 ± 0.4

Table A3. Red blood cell Hg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values in chicks of skua populations breeding at different TAAF districts. Values are mean ± SD. For district abbreviations see Table A1.

Species	**Abb.**	**District**	**Water mass**	**N**	**Hg (µg g^{-1} dw)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Antarctic skua	AS	TA	Antarctic	11	0.51 ± 0.1	-23.5 ± 0.2	11.4 ± 0.5
Subantarctic skua	SSKER	KER	Subantarctic	10	2.31 ± 0.33	-22.7 ± 0.2	10.3 ± 0.2
	SSCRO	CRO	Subantarctic	10	1.66 ± 1.20	-20.22 ± 0.42	10.48 ± 0.66
	SSAMS	AMS	Subtropical	10	3.98 ± 0.77	-17.7 ± 0.4	14.4 ± 0.2

Table A4. Plasma Σ_7PCBs, Σ_{11}OCPs and Σ_{18}POPs (ng g^{-1} ww) and selected organic pesticides in chicks of skua populations breeding at different TAAF districts. Values are mean ± SD. For district abbreviations see Table A1.

Species	Abb.	District	Water mass	HCB	4,4'-DDE	4,4'-DDT	Mirex	Σ_7PCBs	Σ_{11}OCPs	Σ_{18}POPs
Antarctic skua	AS	TA	Antarctic	0.91 ± 0.53 (11)	0.43 ± 0.33 (7)	< LoQ	< LoQ	0.72 ± 0.84 (3)	1.21 ± 0.84 (11)	1.55 ± 1.09 (11)
Subantarctic skua	SSKER	KER	Subantarctic	3.25 ± 0.77 (10)	2.45 ± 0.84 (9)	0.14 ± 0.02 (9)	1.48 ± 0.70 (8)	0.49 ± 0.34 (9)	6.82 ± 2.21 (10)	7.26 ± 2.50 (10)
	SSCRO	CRO	Subantarctic	1.08 ± 0.82 (10)	0.90 ± 1.10 (9)	0.29 ± 0.17 (3)	0.19 ± 0.10 (3)	0.58 ± 0.62 (4)	2.10 ± 2.12 (10)	2.32 ± 2.52 (10)
	SSAMS	AMS	Subtropical	0.21 ± 0.07 (10)	1.33 ± 0.56 (10)	0.20 ± 0.15 (3)	0.31 ± 0.16 (8)	0.18 ± 0.04 (9)	1.90 ± 0.80 (10)	2.06 ± 0.86 (10)

Table A5. Feather Hg concentrations and $\delta^{13}C$ and $\delta^{15}N$ values of actual (2007) and historical samples (1950s-1970s) in selected TAAF penguin species from Terre Adélie (TA, Antarctica) and Crozet Islands (CRO, subantarctic zone).

						Hg (µg g^{-1} dw)			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Species	**Abb.**	**District**	**Water mass**	**Year[1]**	**N**	**Mean ± SD**	**Range**	**Median**	**Mean ± SD**	**Mean ± SD**
Emperor	EP	TA	Antarctic	Historical	14	1.91 ± 0.42	1.32-2.49	1.84	-23.2 ± 0.4	12.7 ± 0.5
				2007	17	1.77 ± 0.37	1.05-2.76	1.71	-23.1 ± 0.3	12.2 ± 0.3
Adélie	AP	TA	Antarctic	Historical	8	2.06 ± 1.41	0.45-4.53	2.03	-24.2 ± 0.7	10.2 ± 1.4
				2007	10	0.66 ± 0.20	0.41-1.06	0.65	-23.4 ± 0.4	10.7 ± 0.6
King	KP	CRO	Subantarctic	Historical	17	2.44 ± 0.51	1.43-3.32	2.30	-20.7 ± 0.6	11.5 ± 0.4
				2007	12	2.89 ± 0.73	2.13-4.47	2.71	-20.8 ± 0.6	11.3 ± 0.5
Macaroni	MP	CRO	Subantarctic	Historical	18	1.88 ± 0.53	1.21-3.18	1.73	-21.1 ± 0.6	10.2 ± 0.7
				2007	12	2.48 ± 0.35	1.82-2.92	2.47	-21.1 ± 0.3	9.7 ± 0.3

[1] The historical sample refers to specimens collected in the TAAF in different years from 1950 to 1977.

Table A6. Plasma Σ_7PCBs, Σ_{11}OCPs and Σ_{18}POPs (ng g^{-1} ww) in chicks of 13 species from the TAAF. The number of individuals with values > LoQ is given in brackets. For district abbreviations see Table A1.

Species	Abb.	District	Water mass	N	Σ_7PCBs	Σ_{11}OCPs	Σ_{18}POPs
Adélie penguin	AP	TA	Antarctic	11	< LoQ	0.18 ± 0.02 (10)	0.18 ± 0.02 (10)
Emperor penguin	EP	TA	Antarctic	10	0.83 ± 1.00 (3)	0.28 ± 0.14 (9)	0.56 ± 0.77 (9)
Snow petrel	SP	TA	Antarctic	10	< LoQ	0.70 ± 0.39 (10)	0.70 ± 0.39 (10)
Thin-billed prion	TBP	KER	Subantarctic	10	< LoQ	1.00 ± 0.37 (10)	1.00 ± 0.37 (10)
Blue petrel	BP	KER	Subantarctic	11	0.10 (1)	1.08 ± 0.66 (11)	1.09 ± 0.68 (11)
Southern rockhopper penguin	SRP	CRO	Subantarctic	10	< LoQ	0.19 ± 0.14 (10)	0.19 ± 0.14 (10)
Macaroni penguin	MP	CRO	Subantarctic	10	< LoQ	0.33 ± 0.30 (10)	0.33 ± 0.30 (10)
King penguin	KP	CRO	Subantarctic	10	0.29 ± 0.14 (3)	0.43 ± 0.31 (10)	0.52 ± 0.36 (10)
Gentoo penguin	GP	CRO	Subantarctic	10	2.55 ± 4.52 (4)	0.21 ± 0.24 (9)	1.34 ± 3.31 (9)
White-chinned petrel	WCP	CRO	Subantarctic	10	2.66 (1)	0.68 ± 0.56 (10)	0.68 ± 0.56 (10)
Northern rockhopper penguin	NRP	AMS	Subtropical	10	0.12 ± 0.01 (2)	0.29 (1)	0.18 ± 0.10 (3)
Yellow-nosed albatross	YNA	AMS	Subtropical	10	0.15 (1)	0.37 ± 0.39 (10)	0.39 ± 0.39 (10)
Amsterdam albatross	AA	AMS	Subtropical	10	2.36 ± 3.16 (6)	2.09 ± 1.62 (10)	3.51 ± 3.56 (10)

Table A7. Red blood cell trace elements concentrations (µg g^{-1} dw) in chicks of 13 TAAF species. The number of individuals with values > LoQ is given in brackets. Values of Ag, As and V were < LoQ in all chicks. For district and species abbreviations see Table A1 and A6, respectively.

Species	District	N	Hg	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
Penguins, albatrosses and petrels													
AP	TA	14	0.20 ± 0.04 (14)	(0)	(0)	(0)	0.95 ± 0.27 (14)	2517 ± 59 (14)	0.16 ± 0.07 (10)	(0)	0.03 ± 0.01 (2)	4.6 ± 1.8 (14)	34.7 ± 21.9 (14)
EP	TA	10	0.37 ± 0.07 (10)	0.30 ± 0.10 (2)	(0)	(0)	0.49 ± 0.05 (10)	2373 ± 80 (10)	(0)	0.05 (1)	(0)	3.1 ± 0.4 (10)	24.6 ± 2.3 (10)
SP	TA	10	0.10 ± 0.03 (10)	0.80 ± 0.47 (9)	(0)	0.02 (1)	0.40 ± 0.11 (10)	2250 ± 102 (10)	0.37 ± 0.18 (7)	(0)	0.02 (1)	14.2 ± 6.0 (10)	27.6 ± 8.7 (10)
TBP	KER	12	0.05 ± 0.01 (12)	(0)	0.13 ± 0.08 (12)	(0)	0.64 ± 0.47 (12)	2177 ± 85 (12)	0.21 ± 0.02 (2)	(0)	0.05 ± 0.01 (2)	33.9 ± 7.6 (12)	30.0 ± 2.0 (12)
BP	KER	11	0.13 ± 0.05 (11)	(0)	0.41 ± 0.19 (11)	(0)	0.47 ± 0.05 (11)	2175 ± 95 (11)	(0)	(0)	(0)	32.9 ± 8.4 (11)	30.4 ± 4.0 (11)
SRP	CRO	10	0.28 ± 0.30 (10)	0.23 (1)	0.04 (1)	(0)	0.78 ± 0.12 (10)	2235 ± 147 (10)	0.26 ± 0.08 (10)	(0)	(0)	22.8 ± 55.0 (10)	27.5 ± 3.2 (10)
MP	CRO	10	0.92 ± 0.16 (10)	(0)	0.03 ± 0.01 (10)	(0)	1.23 ± 0.28 (10)	2306 ± 260 (10)	0.27 ± 0.14 (10)	(0)	(0)	171 ± 50 (10)	20.7 ± 1.6 (10)
KP	CRO	10	0.80 ± 0.11 (10)	(0)	0.03 (1)	0.16 (1)	1.30 ± 0.20 (10)	2337 ± 148 (10)	0.14 ± 0.01 (5)	(0)	0.02 (1)	4.2 ± 0.6 (10)	21.4 ± 1.8 (10)
GP	CRO	10	0.80 ± 0.39 (10)	(0)	(0)	(0)	1.16 ± 0.12 (10)	2437 ± 114 (10)	0.12 ± 0.02 (6)	(0)	(0)	5.2 ± 0.7 (10)	26.1 ± 3.5 (10)
WCP	CRO	11	1.12 ± 0.39 (11)	0.81 ± 0.51 (11)	0.13 ± 0.08 (11)	(0)	0.90 ± 0.14 (11)	2367 ± 72 (11)	0.37 ± 0.15 (11)	(0)	0.23 ± 0.27 (6)	46.0 ± 13.6 (11)	25.2 ± 2.0 (11)
NRP	AMS	10	0.23 ± 0.04 (10)	0.59 ± 0.25 (5)	0.03 ± 0.01 (4)	0.04 (1)	1.08 ± 0.31 (10)	2357 ± 135 (10)	0.44 ± 0.20 (10)	(0)	0.33 ± 0.44 (2)	7.9 ± 2.9 (10)	31.6 ± 5.4 (10)
YNA	AMS	10	0.73 ± 0.45 (10)	0.34 ± 0.09 (8)	0.03 ± 0.01 (2)	1.25 (1)	1.21 ± 0.13 (10)	2057 ± 169 (10)	0.14 ± 0.03 (6)	0.10 (1)	0.03 ± 0.00 (2)	62.8 ± 11.6 (10)	24.6 ± 1.8 (10)
AA	AMS	11	2.66 ± 0.81 (11)	0.31 ± 0.11 (2)	0.05 ± 0.03 (11)	0.03 (1)	1.17 ± 0.07 (11)	1823 ± 188 (11)	(0)	0.07 ± 0.03 (2)	0.03 ± 0.01 (4)	26.9 ± 7.7 (11)	22.7 ± 2.1 (11)
Skuas													
AS	TA	11	0.51 ± 0.10 (11)	1.03 ± 0.97 (11)	(0)	(0)	0.98 ± 0.18 (11)	2397 ± 80 (11)	0.15 ± 0.01 (3)	0.27 ± 0.38 (3)	0.04 (1)	40 ± 17 (11)	23.6 ± 2.0 (11)
SSKER	KER	10	2.31 ± 0.33 (10)	(0)	(0)	(0)	0.61 ± 0.07 (10)	2276 ± 68 (10)	(0)	0.12 (1)	(0)	578 ± 157 (10)	25.4 ± 2.5 (10)
SSCRO	CRO	10	1.66 ± 1.20 (10)	0.23 (1)	(0)	(0)	0.87 ± 0.06 (10)	2201 ± 119 (10)	0.13 ± 0.01 (4)	(0)	(0)	226 ± 82 (10)	22.7 ± 1.5 (10)
SSAMS	AMS	10	3.98 ± 0.77 (10)	0.52 ± 0.21 (10)	(0)	0.05 (1)	1.20 ± 0.14 (10)	2362 ± 89 (10)	0.15 ± 0.07 (7)	0.08 ± 0.03 (3)	0.09 (1)	646 ± 123 (10)	27.1 ± 2.4 (10)

Paper 1

Moulting patterns drive within-individual variations of stable isotopes and mercury in seabird body feathers: implications for monitoring of the marine environment

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METHOD

Moulting patterns drive within-individual variations of stable isotopes and mercury in seabird body feathers: implications for monitoring of the marine environment

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Abstract One major limitation in the use of body feathers of seabirds as a monitoring tool of the trophic structure and contamination levels of marine ecosystems is the degree of heterogeneity in feather chemical composition within individuals. Here, we tested the hypothesis that moulting patterns drive body feather heterogeneity, with synchronous moult minimizing within-individual variations, in contrast to asynchronous feather growth. Chicks of white-chinned petrels *Procellaria aequinoctialis* (representative of bird chicks) and adults of king penguins *Aptenodytes patagonicus* (representative of adult penguins) that moult their body feathers synchronously showed very low within-individual variations in their feather $\delta^{13}C$ and $\delta^{15}N$ values and mercury (Hg) concentrations. By contrast, body feathers of adults of Antarctic prions *Pachyptila desolata* (representative of adult seabirds with asynchronous feather growth during a protracted moult) presented much higher within-individual variances for the three parameters. These findings have three important implications for birds presenting a synchronous body moult. (1) They suggest that all body feathers from the same individual have identical $\delta^{13}C$ and $\delta^{15}N$ values and Hg content. (2) They predict negligible within-individual variations in the body feather values of other useful stable isotopes, such as $\delta^{2}H$ and $\delta^{34}S$, as well as in the concentrations of other compounds that are deposited in the keratin structure. (3) Analysis of one or any number of pooled body feathers is equally representative of the individual. In conclusion, we recommend that long-term routine monitoring investigations focus on birds presenting synchronous rather than asynchronous moult of body feathers both in marine and terrestrial environments. This means targeting chicks rather than adults and, for seabirds, penguins rather than adults of flying species.

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Introduction

Feathers are used extensively in avian biology, chemoecology and toxicology because they can be easily collected and are metabolically inert after synthesis, thus preserving their chemical composition almost indefinitely. For instance, stable isotope analyses of feathers have provided insights into many aspects of avian ecology (Inger and Bearhop 2008; Hobson 2011), and feathers are considered as excellent non-destructive tools for monitoring contaminants, including heavy metals and persistent organic pollutants (Burger 1993; García-Fernández et al. 2013). More recently, the application of the stable isotope method to ecotoxicological studies has granted insight into patterns of contamination among sites, species and individuals (e.g. Ofukany et al. 2012; Ramos et al. 2013). Feathers from museum collections have been further used to depict historical changes in birds' foraging ecology and contaminant exposure (Vo et al. 2011; Jaeger and Cherel 2011).

Ideally, a monitoring tool must show little within- and between-individual variations in the levels of targeted compounds in order to facilitate the statistical description of spatio-temporal trends within a population. Indeed, a main limitation in the use of feathers is within-individual heterogeneity both between and within feather types. For

example, changes in foraging habitat or diet during moult lead to large variations in feather $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Jaeger et al. 2010), and feathers that grow at different times present different mercury (Hg) concentrations, as the Hg body pool is progressively depleted during the moult (Furness et al. 1986; Braune and Gaskin 1987). For both scientific and ethical reasons, body feathers are generally considered as the best feather type to sample (Furness et al. 1986; Jaeger et al. 2009), but few investigations looked at their stable isotope values and contaminant levels of within-individual heterogeneity (Thompson et al. 1993; Bond and Diamond 2008; Jaeger et al. 2009, 2010; Brasso et al. 2013). Since body feathers grow asynchronously during the whole moulting period, a main recurrent problem is that the precise timing of synthesis of a given body feather remains unknown. However, the temporal drawback can be eluded in the case of synchronous or almost synchronous moult of body feathers. Indeed, assuming a constant growth rate for all body feathers, a synchronous growth theoretically means that all body feathers should have the same chemical composition and thus should show identical stable isotope values and contaminant levels.

Here, we tested this hypothesis by measuring $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations in four body feathers from the same individuals of three groups of marine birds showing different moulting patterns. Seabirds are useful organisms to biomonitor marine ecosystems, because they are long-lived animals that prey at the top of the marine food webs (Furness and Camphuysen 1997; Burger and Gochfeld 2004). Selected models were:

1. Chicks of white-chinned petrels *Procellaria aequinoctialis*. Chicks of this species were selected as representative of bird chicks, which present a complete moult with body feathers growing almost synchronously towards the end of the chick-rearing period. White-chinned petrel chicks have the advantage of showing significant between-individual variations in their feather stable isotope values (Jaeger et al. 2010), being thus a potentially good model to compare feather heterogeneity between and within individuals.
2. Adults of Antarctic prions *Pachyptila desolata*. Adults of this species were selected as representative of adults of flying seabirds that disperse after breeding and moult far away from their breeding grounds during the inter-nesting period. Moult is sequential and generally protracted over several weeks to months (Bridge 2006). Antarctic prions breed within the Southern Ocean and their feather $\delta^{13}C$ values indicate moulting primarily in northern warmer waters (Cherel et al. 2006).
3. Adults of king penguins *Aptenodytes patagonicus*. Adults of this species were selected as representative of penguins, which present a unique moulting pattern among birds. Penguins renew their whole plumage while fasting ashore, because transient reduction in thermal insulation during moult prevents them from going at sea. Consequently, all their body feathers grow simultaneously at the expense of energy reserves that are built up during the pre-moulting foraging period of hyperphagia at sea (Groscolas and Cherel 1992; Cherel et al. 1994).

As body feathers grow simultaneously in white-chinned petrel chicks and king penguin adults, but not in Antarctic prion adults, our driving hypothesis was that feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations would present lower within- than between-individual variations in the two former groups and would show an opposite pattern in the latter species. The main consequence of these moulting strategies is that chicks and penguins should be more suitable avian models than adults of flying species for long-term monitoring of the marine environment.

Materials and methods

Body feathers from white-chinned petrel chicks and king penguin adults were collected on Possession Island (46°30′S, 51°45′E), Crozet Archipelago in 2007 and 2005, respectively. King penguin adults were sampled during their annual moult, while well-feathered white-chinned petrel chicks were sampled at the end of the chick-rearing period. Antarctic prion adults were collected on the Kerguelen Islands (49°21′S, 70°18′E) during the 2011–2012 austral summer. Birds were found dead or dying after being trapped in the vegetation (*Acaena adscendens*); they were stored at −20 °C until dissection at the Centre d'Etudes Biologiques de Chizé, France. For all species, several whole body feathers per individual were pulled out from the lower back (dorsal tract) and stored dry in sealed plastic bags until analysis at the University of La Rochelle, France. Prior to chemical analysis, whole feathers were cleaned as described in Blévin et al. (2013) and then oven-dried for 48 h at 50 °C. Every whole feather was homogenized by cutting it with scissors into small fragments, and a subsample of ~0.3 mg was packed into tin containers for stable isotope analysis. The relative abundance of carbon and nitrogen isotopes was determined with a continuous-flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). Results are presented in the usual δ notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated measurement errors <0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. The remaining homogenized subsample of every feather was then analysed

for total Hg in an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254) following Blévin et al. (2013). Whenever possible, Hg analysis was repeated twice, and the relative standard deviation between runs calculated (<10 % for all samples). Accuracy was checked using a certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 μg g^{-1} dry weight). Our measured values were 0.268 ± 0.022 μg g^{-1} dry weight ($n = 14$). Blanks were analysed at the beginning of each set of samples, and the detection limit of the method was 0.005 μg g^{-1} dry weight. Data of Hg concentrations are presented relative to the dry weight (dw).

Statistical analyses were performed using R 2.15.1 (R Core Team 2012). Linear mixed-effect models were used in order to test the repeatability of feather stable isotope values and Hg concentrations within individuals. Random effect models with the individual as a random intercept were constructed for the three groups of birds. The variance explained by the model (d), i.e. the between-individual variance, and the residual variance (σ) were used to calculate the intra-class correlation coefficient (ICC) as $d^2/(d^2 + \sigma^2)$, which is a measure of repeatability (Nakagawa and Schielzeth 2010). ICC ranges from 0 to 1, with values close to one meaning that most of the variance is explained by between-individual differences. Stable isotope values and Hg concentrations are mean ± standard deviation (SD).

Results

Feather stable isotope values and Hg concentrations were measured in four different body feathers from ten white-chinned petrel chicks, ten Antarctic prion adults and seven king penguin adults (Table 1; Fig. 1). Individual SD was very low and ICC very high for feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations of white-chinned petrel chicks and king penguin adults (Tables 1, 2). By contrast, Antarctic prion adults showed large within-individual variations, i.e. high SD and low ICC in feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations. In Antarctic prion adults, individual ranges of stable isotopes values were as large as 4.9 and 5.6 ‰ in feather $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Noticeably, while feather Hg concentrations were within the same order of magnitude for the three groups of birds (overall individual range: 1.1–3.9 μg g^{-1} dw), CV was much lower in individual white-chinned petrel chicks (3–9 %) and king penguin adults (<1–8 %) than in Antarctic prion adults (15–60 %).

Discussion

To the best of our knowledge, this study is the first to compare the levels of heterogeneity in stable isotope values and contaminant concentrations of body feathers both within and between individuals in relation to different moulting patterns. The results verified the initial hypothesis stating that a synchronous moult leads to lower within- than between-individual variations in feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations, while asynchronous feather growth induces higher levels of feather heterogeneity within individual birds. Adult moult is potentially of variable duration according to sex, age, breeding status, species, phylogeny and environmental constraints. Hence, the synchronous/protracted moult hypothesis merits further investigations focusing on slow-moulting birds (e.g. albatrosses, Bridge 2006), species undergoing two moults per year (e.g. terns, Burger et al. 1992) and species with complex moulting patterns (e.g. alcids, Bridge 2004).

Feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations showed low variances and high ICC, thus indicating low levels of within-individual variations in white-chinned petrel chicks and king penguin adults. Noticeably, SD of $\delta^{13}C$ and $\delta^{15}N$ values was close to the accuracy of the isotopic method. These findings have three important implications.

1. They suggest that all body feathers that grow simultaneously have the same $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations.
2. When considering the overall feather chemical composition, they predict negligible within-individual variations in other useful stable isotope values of keratin, such as $\delta^{2}H$ and $\delta^{34}S$ (Hobson 2011; Ramos et al. 2013) and in the concentrations of other compounds that are deposited in the keratin structure, such as trace metals and metalloids, persistent organic pollutants and hormones (Burger 1993; Bortolotti et al. 2009; García-Fernández et al. 2013). This prediction is not verified for at least one group of molecules, the pigments, which can vary qualitatively and quantitatively from one body feather to the other (Stettenheim 2000). It also merits further investigations for those compounds that show a time-dependent deposition in feathers, such as steroid hormones (Bortolotti 2010).
3. Analysis of one to several pooled body feathers will provide identical stable isotope values and concentrations in various chemical compounds, meaning that any quantity of body feathers that grow simultaneously is equally representative of the individual. This is particularly relevant for studies including several elements and molecules, because some measurements necessitate only a single body feather (e.g. Hg, $\delta^{13}C$ and $\delta^{15}N$), but others require much more material, and hence several feathers (e.g. organic pollutants; García-Fernández et al. 2013).

Table 1 Feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations in white-chinned petrel chicks, Antarctic prion adults and king penguin adults

Id	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			Hg (μg g^{-1} dw)			
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	CV (%)
White-chinned petrel chicks										
1	−21.7	−21.0	−21.3 ± 0.3	11.4	12.2	11.9 ± 0.3	1.22	1.51	1.35 ± 0.12	8.86
2	−20.7	−20.6	−20.6 ± 0.1	12.5	12.9	12.7 ± 0.2	2.06	2.20	2.11 ± 0.06	2.95
3	−23.0	−22.7	−22.8 ± 0.1	10.8	11.1	11.0 ± 0.1	1.01	1.22	1.09 ± 0.09	8.35
4	−20.6	−20.5	−20.6 ± 0.1	12.5	13.2	12.8 ± 0.3	1.93	2.33	2.18 ± 0.17	7.91
5	−22.3	−21.8	−22.0 ± 0.2	11.7	11.9	11.8 ± 0.1	2.47	2.93	2.72 ± 0.19	6.99
6	−21.5	−21.0	−21.2 ± 0.2	12.0	12.3	12.2 ± 0.1	1.47	1.74	1.59 ± 0.12	7.70
7	−20.3	−20.1	−20.2 ± 0.1	12.7	13.1	12.9 ± 0.2	2.85	3.42	3.11 ± 0.25	8.12
8	−21.6	−21.4	−21.5 ± 0.1	12.2	12.3	12.2 ± 0.1	1.61	1.81	1.74 ± 0.09	5.08
9	−22.1	−22.0	−22.1 ± 0.1	11.3	11.4	11.4 ± 0.1	1.28	1.40	1.34 ± 0.06	4.13
10	−21.1	−20.7	−20.9 ± 0.2	12.7	13.0	12.8 ± 0.1	2.40	2.73	2.59 ± 0.14	5.32
Antarctic prion adults										
1	−18.7	−18.4	−18.6 ± 0.2	8.8	9.4	9.1 ± 0.2	1.73	3.79	2.87 ± 1.07	37.3
2	−18.4	−16.9	−18.0 ± 0.7	9.7	15.2	11.7 ± 2.4	1.35	2.89	2.39 ± 0.71	29.7
3	−21.6	−16.7	−18.6 ± 2.1	9.0	13.0	10.9 ± 1.7	1.13	3.45	2.40 ± 0.96	39.9
4	−19.0	−17.9	−18.4 ± 0.5	8.5	10.8	9.2 ± 1.1	1.14	2.86	1.86 ± 0.78	42.0
5	−19.1	−18.6	−18.8 ± 0.2	8.2	9.4	8.6 ± 0.6	1.00	1.77	1.35 ± 0.40	29.8
6	−21.3	−18.3	−19.4 ± 1.3	8.3	9.9	9.3 ± 0.7	1.68	2.42	2.13 ± 0.36	15.3
7	−18.6	−16.4	−17.9 ± 1.0	8.4	14.0	10.0 ± 2.7	1.53	3.11	2.26 ± 0.84	37.3
8	−18.3	−17.8	−18.0 ± 0.2	9.1	10.1	9.7 ± 0.4	0.97	1.59	1.30 ± 0.25	19.6
9	−18.7	−16.3	−17.4 ± 1.2	9.1	11.3	10.4 ± 1.0	1.47	4.89	2.70 ± 1.61	59.8
10	−19.3	−17.8	−18.5 ± 0.6	8.6	10.8	9.4 ± 1.0	2.40	4.77	3.95 ± 1.08	27.3
King penguin adults										
1	−21.4	−21.0	−21.2 ± 0.2	10.7	11.1	11.0 ± 0.1	2.18	2.36	2.27 ± 0.08	3.52
2	−20.1	−19.8	−19.9 ± 0.1	11.3	11.7	11.5 ± 0.2	3.78	3.82	3.80 ± 0.02	0.54
3	−21.2	−21.0	−21.1 ± 0.1	11.3	11.5	11.4 ± 0.1	2.07	2.36	2.22 ± 0.14	6.38
4	−21.5	−21.2	−21.4 ± 0.1	10.7	11.1	10.8 ± 0.2	2.78	2.97	2.88 ± 0.08	2.79
5	−20.6	−20.3	−20.5 ± 0.1	11.0	11.1	11.1 ± 0.1	2.48	2.74	2.57 ± 0.12	4.69
6	−20.0	−19.8	−19.9 ± 0.1	12.1	12.3	12.2 ± 0.1	3.15	3.77	3.41 ± 0.28	8.20
7	−22.9	−22.1	−22.3 ± 0.4	11.1	11.6	11.4 ± 0.2	2.33	2.41	2.37 ± 0.04	1.84

Values are mean ± SD of four feathers per individual bird

CV coefficient of variation, *Id* individual

In contrast to white-chinned petrel chicks and king penguin adults, the amount of variability in feather $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations within individuals was high in Antarctic prion adults. Such high levels of heterogeneity were previously found in the feather isotopic values of adults of light-mantled (*Phoebetria palpebrata*) and wandering (*Diomedea exulans*) albatrosses (Jaeger et al. 2009, 2010) and in body feather Hg concentrations of adults of Arctic (*Sterna paradisaea*) and common terns (*S. hirundo*) and of Leach's storm petrels (*Oceanodroma leucorhoa*) (Bond and Diamond 2008). The within-individual variation of adult flying birds likely results from body feathers being synthesized and replaced at different times during the moulting period (e.g. Battam et al. 2010). This heterogeneity reflects the birds' movements within different water masses and associated dietary shifts (Jaeger et al. 2010), together with changes in body burdens of Hg (Furness et al. 1986; Braune and Gaskin 1987) during the protracted moult. A positive aspect of feather variability is that measuring the isotopic values of several body feathers per individual can provide valuable information on the foraging strategies of adult birds during the moulting period (Jaeger et al. 2009, 2010). A negative aspect is that it complicates the use of body feathers as an effective monitoring tool because increasing variability can blur temporal and spatial trends. Hence, in a first step, within-individual heterogeneity must be accurately quantified, as well as the minimum number of body feathers to sample, as described for example by Jaeger et al. (2009) and Brasso et al. (2013). In a second step, body feathers of a given individual can be pooled in order to perform a unique measurement per bird. However, uncertainties in the biological interpretation of the data will remain, since, in most cases, practical reasons preclude determining the precise timing of synthesis

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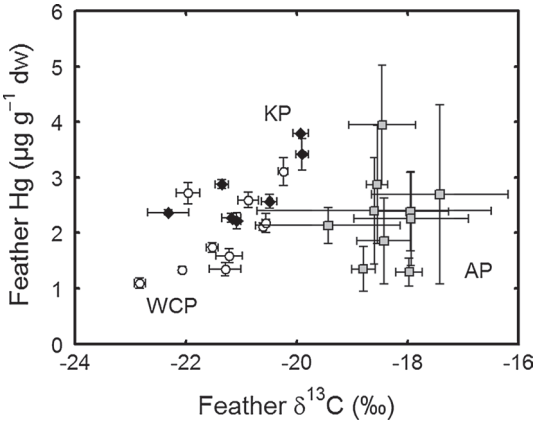

Fig. 1 Simultaneously moulting white-chinned petrel chicks (*WCP*; *white circles*) and king penguin adults (*KP*; *black diamonds*) show smaller SD in both feather $\delta^{13}C$ and Hg values than sequentially moulting Antarctic prion adults (*AP*; *grey squares*). Values are mean ± SD of four feathers per individual bird

of the sampled body feathers in relation to the food and feeding ecology of the individuals. Minimizing heterogeneity related to analytical procedure also requires thorough feather homogenisation.

We therefore recommend that long-term routine monitoring investigations on the trophic structure and contamination levels of ecosystems focus on birds presenting synchronous rather than asynchronous moult of body feathers. This means targeting chicks rather than adults (Blévin et al. 2013; this study), and, in the Southern Hemisphere, adult penguins rather than adult flying seabirds (Carravieri et al. 2013; this study). Chicks may present two disadvantages: (i) they may show low magnitude variation in feather $\delta^{15}N$ (not $\delta^{13}C$) in relation to varying chick growth rates (~0.6 ‰, Sears et al. 2009), and (ii) museum specimens include only a few well-feathered chicks, thus precluding determining historical changes. Nevertheless, chicks present several advantages over other age classes.

1. Most seabird chicks can be easily handled since they remain on land, while being fed exclusively by their parents. In addition, chick feathers can be sampled before fledging with minimum disturbance (e.g. during the annual ringing session in a long-term study colony).
2. The food of chicks and the foraging ecology of parent seabirds can be investigated by collecting stomach samples and using bio-logging. Hence, feather stable isotope values and contaminant levels can be related to the feeding ecology of the animals. Since adult seabirds are central place foragers when breeding, stable isotopes and contaminants in chick feathers likely represent primarily the local environment.
3. Chick moult is easy to study and the time window integrated by chick feathers is well-defined, because growth of body feathers takes place on land in the mid- to the second half of the chick-rearing period (Bost and Jouventin 1991; Phillips and Hamer 2000).
4. Working on chicks minimizes the temporal mismatch resulting from different integration times between feather stable isotopes and some contaminants, such as Hg (Bond 2010). Feather $\delta^{13}C$ and $\delta^{15}N$ values reflect the feeding ecology of the birds at the time of feather synthesis (Hobson and Clark 1992; Bearhop et al. 2002), while feathers integrate Hg accumulated in internal tissues during two successive moults (Furness et al. 1986). In chicks, this means the second part of the chick-rearing period (see above) versus a slightly longer period corresponding to the end of down growth (initial chick moult) to the beginning of body feather growth (Stewart et al. 1997), respectively.

In agreement with Stewart et al. (1997) and Burger and Gochfeld (2004), the present work provides new evidence showing that chick feathers are a useful avian tool for routine biomonitoring of the trophic structure and contaminant bioavailability in the marine environment. By targeting species with different breeding locations and chick-rearing periods (for example summer vs. winter breeders), different, but well-defined spatio-temporal scales of the marine environment can be investigated. The present work focuses on seabirds, but it can be generalized to water birds and terrestrial species, since a synchronous or almost synchronous moult of body feathers in chicks before fledging is a general pattern among avian species.

Table 2 Variance parameters and intra-class correlation coefficients (ICC) of linear mixed-effects models with the individual as a random intercept (random effects models) for white-chinned petrel chicks, Antarctic prion adults and king penguin adults

Species	$\delta^{13}C$			$\delta^{15}N$			Hg		
	d^2	σ^2	ICC	d^2	σ^2	ICC	d^2	σ^2	ICC
White-chinned petrel chicks ($n = 10$)	0.64	0.03	0.96	0.44	0.04	0.92	0.45	0.02	0.96
Antarctic prion adults ($n = 10$)	0.07	0.99	0.07	0.40	1.99	0.17	0.40	0.81	0.33
King penguin adults ($n = 7$)	0.72	0.03	0.96	0.21	0.02	0.91	0.37	0.02	0.95

d, variance explained by the model, here the between-individual variance; σ, residual variance

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Paper 2

Penguins as bioindicators of mercury contamination in the Southern Ocean: birds from the Kerguelen Islands as a case study

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Penguins as bioindicators of mercury contamination in the Southern Ocean: Birds from the Kerguelen Islands as a case study

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HIGHLIGHTS

- Hg contamination was evaluated in 4 species of penguins at the Kerguelen Islands.
- Adults displayed significantly higher Hg levels than chicks in all species.
- Species and feeding habits (δ15N) were major determinants of Hg levels.
- Dietary specialisation was essential in explaining Hg levels in gentoo penguins.
- Penguins are reliable bioindicators of Hg contamination in the Southern Ocean.

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ABSTRACT

Seabirds have been used extensively as bioindicators of mercury (Hg) contamination in the marine environment, although information on flightless species like penguins remains limited. In order to assess the use of penguins as bioindicators of Hg contamination in subantarctic and Antarctic marine ecosystems, Hg concentrations were evaluated in the feathers of the four species that breed on the Kerguelen Islands in the southern Indian Ocean. Compared to other seabirds, adult Kerguelen penguins had low to moderate feather Hg concentrations, with an average ranging from 1.96 ± 0.41 μg g^{-1} dry weight in the southern rockhopper penguin to 5.85 ± 3.00 μg g^{-1} dry weight in the gentoo penguin. The species was a major determinant of Hg contamination, with feather Hg concentrations being lower in the oceanic species (king and crested penguins) than in the coastal one (gentoo penguin). In all species however, feather Hg concentrations were higher in adults than in chicks, reflecting the different periods of Hg bioaccumulation in the internal tissues of the two age classes. The relationship between adult penguin trophic ecology and Hg burdens was investigated using stable isotopes. Feeding habits (reflected by $\delta^{15}N$ values) had a greater effect on adult feather Hg concentrations when compared to foraging habitats (reflected by $\delta^{13}C$ values), indicating Hg biomagnification in Kerguelen neritic and oceanic waters. Dietary preferences were crucial in explaining individual feather Hg concentrations, as highlighted by intra-specific variation in Hg levels of gentoo penguins sampled at two different breeding sites of the archipelago. Penguins appear to reflect Hg bioavailability reliably in their foraging environment and could serve as efficient bioindicators of Hg contamination in the Southern Ocean on different spatial and temporal scales.

1. Introduction

While occurring naturally, mercury (Hg) is a pervasive environmental contaminant that negatively impacts humans and wildlife (Bond and Diamond, 2009; Scheuhammer et al., 2007). Over centuries a vast range of human activities have increased emissions to the atmosphere, modifying the cycling of Hg on the world scale (Fitzgerald et al., 2007; Selin, 2009). Aquatic environments, including marine ecosystems, are major repositories of natural and anthropogenic Hg. Hence, Hg is widely distributed in the World Ocean as a consequence of both long-range atmospheric transport and deposition (Ebinghaus et al., 2002; Fitzgerald et al., 1998). Despite being free of industrial sources of contamination and scarcely affected by local anthropogenic pollution, the Southern Ocean presents some unique features in the distribution of the different Hg species (Cossa et al., 2011), including elevated levels of contamination in some biota, especially top predators (Anderson et al., 2009; Bargagli et al., 1998; Muirhead and Furness, 1988). Indeed, Hg is known to bioaccumulate in the tissues of living organisms and to biomagnify within food webs, leaving top predators at risk of high contamination levels through food intake

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(Furness and Camphuysen, 1997; Morel et al., 1998). Top consumers include seabirds that have been identified as effective Hg sentinels in the marine environment (e.g., Burger and Gochfeld, 2004; Furness, 1993). In this context, seabird feathers have proved to be a valuable tissue, as they represent the main route of Hg excretion in birds (e.g., Braune and Gaskin, 1987; Monteiro and Furness, 1995) and can be easily collected during nesting from both chicks and breeding adults.

Previous investigations on Hg contamination of seabirds from the Southern Ocean essentially focused on flying species, mainly of the order Procellariiformes (Anderson et al., 2009; Becker et al., 2002; Bocher et al., 2003). By contrast, very little is known about the Hg exposure of diving seabirds like penguins (Bargagli et al., 1998; Becker et al., 2002; Scheifler et al., 2005). Penguins are less Hg contaminated than some other seabirds, but they present interesting ecological and practical advantages over flying species to investigate Hg contamination within Antarctic and subantarctic food webs. Firstly, unlike most albatrosses and petrels that disperse in northern waters during the nonbreeding period (BirdLife International, 2004), Antarctic and subantarctic penguins are restricted to the Southern Ocean all year long (Ballard et al., 2010; Thiebot et al., 2011a, 2011b, 2012). Penguins are thus truly representative of the level of contamination of Antarctic and subantarctic ecosystems. Secondly, depending on species, penguins forage at different depths of the water column, namely the epi-, meso-pelagic and benthic zones that are known to present heterogeneous Hg concentrations and Hg species distributions (Cossa et al., 2011; Fitzgerald et al., 2007; Thompson et al., 1998). Thirdly, penguins renew their whole plumage annually over a 2–4 week period on land (Adams and Brown, 1990; Cherel et al., 1994), thus contrasting with most other birds that present a prolonged, sequential moult leading to higher Hg concentrations in the earlier than in the later growing feathers (Furness et al., 1986). Hence, penguins appear to be good models to evaluate Hg contamination in their foraging environment, but this has yet to be proved conclusively.

The main objective of the present study was to assess the use of penguins as bioindicators of Hg contamination in marine ecosystems of the Southern Ocean. The following predictions were tested on the penguin assemblage from the subantarctic Kerguelen Islands where four species live in sympatry.

1) As already depicted in other seabirds (Anderson et al., 2009; Becker et al., 2002; Blévin et al., 2013), the diet and foraging ecology of penguins should play an important role in explaining feather Hg levels, because ingestion of food is the main route of Hg exposure in birds. As Kerguelen penguin species display contrasted feeding ecology (Table 1), feather Hg concentrations should show important inter-specific differences. The respective effects of habitats and diets were tested by using the isotopic niche as a proxy of the trophic niche of the species, with the ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) reflecting their foraging habitats and trophic positions, respectively. The isotopic method was already validated in the area, with seabird $\delta^{13}C$ values indicating their latitudinal foraging grounds and depicting offshore versus inshore consumers and their $\delta^{15}N$ values increasing with trophic level (Cherel and Hobson, 2007; Cherel et al., 2007, 2010; Jaeger et al., 2010). Taking into account the penguins' foraging ecology (Table 1), we make the following predictions. Firstly, feeding habitat ($\delta^{13}C$) should shape seabird Hg contamination, because Hg is not homogeneously distributed in marine ecosystems (Cossa et al., 2011; Hammerschmidt and Bowman, 2012). For example, benthic foragers should have higher feather Hg concentrations than pelagic foragers in relation to the substantial production of Me–Hg in coastal marine sediments (Fitzgerald et al., 2007). Secondly, penguins with the highest trophic positions ($\delta^{15}N$) should show the highest feather Hg concentrations, because Hg biomagnifies within marine food webs (e.g., Selin, 2009).
2) Between-year variation of Hg levels is an issue rarely investigated in seabirds. Taking into account that (i) penguin feeding habits are comparable in the breeding and non-breeding seasons (Thiebot et al., 2011a, 2011b, 2012); (ii) penguin feather Hg temporal integration is constant (one year); and (iii) the Kerguelen Islands are remote from anthropogenic Hg sources; no variation in feather Hg levels should be detected on a short temporal scale (two following years).
3) As already found in birds (Burger, 1993), Hg concentrations in the feathers should be higher in adult penguins than in chicks at fledging, mainly because: (i) the time interval of Hg accumulation is longer in adults than in chicks (~12 months of inter-moult period for adults and the 3–9 months of rearing period in chicks, depending on species) and (ii) Hg can bioaccumulate in internal tissues of long-lived animals over their whole life span.

The present article is the first exploratory step of a wider investigation on penguins as bioindicators of Hg contamination in the Southern Ocean. In the second step, we will focus on penguins breeding at different locations in order to highlight potential geographic

Table 1
Foraging ecology of penguins during the breeding and non-breeding periods at the Kerguelen Islands.

Species	Foraging habitat		Chick diet	References
Colony location	Breeding season (horizontal; vertical)	Non-breeding season (horizontal; vertical)		
King penguin				
Ratmanoff	Polar Frontal Zone (oceanic; epi-mesopelagic)	Unknown (likely in cold oceanic waters; epi-mesopelagic)	Pelagic fish	Bost et al. (2002), Cherel et al. (2010)
Macaroni penguin				
Cap Cotter	Eastwards off Kerguelen in the Polar Frontal Zone (oceanic; epipelagic)	Polar Frontal Zone (oceanic)	Pelagic crustaceans and fish	Cherel et al. (2010), Thiebot et al. (2011a, 2011b)
Southern rockhopper penguin				
Mayes, Morbihan Bay	Morbihan Bay (neritic; pelagic and benthic)	Subantarctic and Polar Frontal Zones (oceanic; epipelagic)	Pelagic crustaceans and fish	Tremblay and Cherel (2000, 2003), Cherel et al. (2010), Thiebot et al. (2012)
Gentoo penguin				
Ratmanoff (open sea)[a]	Eastwards off Kerguelen (neritic; benthic and pelagic);	Resident all year long	Benthic fish and pelagic crustaceans	Lescroël et al. (2004), Lescroël and Bost (2005)
Penn Island, Morbihan Bay (closed sea)	Morbihan Bay (coastal; pelagic)	Resident all year long	Pelagic crustaceans	Lescroël et al. (2004), Lescroël and Bost (2005)

[a] Cape Ratmanoff is close to Cape Estacade and diet of gentoo penguins is similar in both sites.

variation of Hg levels over a large latitudinal gradient, from the subtropics to Antarctica. In the third and final step, we will assess historical trends of penguin Hg contamination by comparing actual feather concentrations with those of museum specimens dating as far back as the 1950s.

2. Materials and methods

2.1. Study site, species and field collections

Fieldwork was carried out during the 2006–2007 austral summer on the Kerguelen Islands (49°21′S, 70°18′E), which are located in the southern part of the Polar Frontal Zone, in the immediate vicinity of the Polar Front (Park and Gamberoni, 1997). The Kerguelen assemblage of penguins is composed of four species, namely the king *Aptenodytes patagonicus* (KP), macaroni *Eudyptes chrysolophus* (MP), southern rockhopper *Eudyptes chrysocome filholi* (SRP) and gentoo penguins *Pygoscelis papua* (GP) (Table 1). Sampling was conducted at different locations of the archipelago, depending on the species breeding sites. Since GP have different foraging strategies depending on the colony location, they were sampled in two contrasting marine environments, an enclosed bay (Penn Island, located in the large Baie du Morbihan) and an open water site (Estacade) (Table 1).

Penguin moult involves two distinct processes, with new feather synthesis and old feather loss overlapping in mid-moult (Cherel et al., 1994). Thus both new and old feathers from the same individual adult penguins were collected in mid-moult, in order to evaluate potential inter-annual variation of adult penguins Hg exposure at the individual scale. Old and new feathers refer to moults that occurred during the 2005–2006 and 2006–2007 austral summers (hereafter called 2006 and 2007), respectively. Feathers (2007 moult) were also sampled from chicks at fledging (i.e. at the end of the breeding season) and, for KP only, from moulting immature birds (i.e. young birds after their first year at sea). Between 6 and 10 body feathers per individual were pulled out and then stored dry in sealed plastic bags until analysis at the University of La Rochelle, France.

2.2. Sample analyses

Prior to chemical analysis, feathers were cleaned to remove surface lipids and contaminants using a 2:1 chloroform:methanol solution followed by two successive methanol rinses. After cleaning, body feathers were oven dried for 48 h at 50 °C. In a first step, an individual feather per penguin was analysed for total Hg in an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254) following Blévin et al. (2013). Since almost all Hg is under organic form in feathers, total Hg approximates the amount of feather methyl-Hg (Bond and Diamond, 2009; Thompson and Furness, 1989). All analyses were repeated 2–3 times until having a relative standard deviation < 10%. Accuracy was checked using a certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 μg g^{-1} dry mass). Our measured values were 0.226 ± 0.003 μg g^{-1} dry mass, $n = 7$. Blanks were analysed at the beginning of each set of samples and the detection limit of the method was 0.005 μg g^{-1} dry mass. Data of Hg concentrations are presented relative to the dry weight (dw).

In a second step, an individual feather per adult penguin was homogenized by cutting it with scissors into small fragments, weighed (~0.3 mg) with a microbalance and packed into tin containers. The relative abundance of carbon and nitrogen isotopes were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific Flash EA 1112). Results are presented in the usual notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated measurement errors < 0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

2.3. Statistical analysis

Statistical tests were performed using R 2.15.1 (R Core Team, 2012) mainly following Crawley (2007). In univariate unifactorial analyses, all data were first checked for normality and homogeneity of variances by means of Shapiro–Wilk and Bartlett tests, respectively. Depending on the results, parametric or non-parametric tests were used and followed by multiple comparisons tests. Relationships between feather Hg concentrations and continuous explanatory variables (feather $\delta^{13}C$ and $\delta^{15}N$ values) were tested using Pearson or Spearman correlation rank tests.

Multifactorial analyses were used to test multiple alternative hypotheses on the influence of species, foraging habitat and trophic level (inferred from feather $\delta^{13}C$ and $\delta^{15}N$ values, respectively) on feather Hg concentrations. Generalized linear models (GLM) with a normal distribution and an identity-link function were constructed as follows: log-transformed Hg concentrations as the response variable, species as a categorical explanatory variable and feather $\delta^{13}C$ and $\delta^{15}N$ values as continuous explanatory variables. Biologically relevant models were constructed incorporating the different variables and their interactions. Continuous variables ($\delta^{13}C$ and $\delta^{15}N$) that were significantly correlated were not included in the same models. Model selection was based on Akaïke's Information Criteria adjusted for small sample sizes (AIC_c). The model with the lowest AIC_c value was considered to be the most accurate. Models with AIC_c values differing by less than 2 have a similar level of support in the data, and the model including the least number of parameters was regarded as the most accurate, according to the principle of parsimony (Burnham and Anderson, 2002). Overall model support was assessed using Akaïke weights (w_i), following Johnson and Omland (2004) and model fit was checked by residual analysis.

A significance level of $\alpha < 0.05$ was used for all tests, both in unifactorial and multifactorial analysis. Values are means ± SD.

3. Results

3.1. Influence of species and foraging ecology on Hg concentrations in adult penguins

In a first step, unifactorial analyses were used to test interspecific differences in feather Hg concentrations and stable isotopes signatures and their relationships in adult Kerguelen penguins. Feather Hg concentrations of adult penguins were significantly different between species in 2007 (Kruskal–Wallis, $H = 17.6$, $p = 0.001$, $n = 48$). KP, MP and SRP presented similar feather Hg levels, whereas GP were significantly more contaminated and showed a much higher variance (Table 2). Feather $\delta^{13}C$ and $\delta^{15}N$ values varied significantly within the penguin community (Kruskal–Wallis, $H = 35.5$ and $H = 36.5$ for $\delta^{13}C$ and $\delta^{15}N$, respectively, both $p < 0.0001$, $n = 48$), defining three non-overlapping isotopic niches, with identical isotopic signatures of the closely-related MP and SRP (Table 2).

When individual data from the four penguin species were pooled (data not shown), feather Hg concentrations were significantly and positively correlated to both feather $\delta^{13}C$ and $\delta^{15}N$ values (Spearman correlation, $\rho = 0.42$ and 0.50, $p = 0.003$ and 0.0003, respectively, both $n = 48$). Feather $\delta^{13}C$ and $\delta^{15}N$ values were also significantly and positively correlated (Pearson correlation, $r = 0.79$, $p < 0.0001$, $n = 48$).

In a second step, multifactorial analyses were used to disentangle the influence of species, foraging habitat ($\delta^{13}C$) and trophic level ($\delta^{15}N$) on feather Hg concentrations of adult penguins. Since feather $\delta^{13}C$ and $\delta^{15}N$ values were correlated, models including both variables were not included in the set of candidate models (Table 3).

Table 2
Feather Hg concentrations (μg g^{-1} dw) and $\delta^{13}C$ and $\delta^{15}N$ values (‰) of Kerguelen penguins in 2006 and 2007. Values are means ± SD (with ranges in parentheses for Hg).

Species	Feather	Year	*n*	Hg[a]	$\delta^{13}C$[a]	$\delta^{15}N$[a]
King penguin						
Adults	New	2007	12	2.22 ± 0.59 (1.45–3.21) A	−21.29 ± 0.46 A	10.90 ± 0.18 A
Adults	Old	2006	12	2.17 ± 0.52 (1.22–3.14)		
Immatures	New	2007	12	1.79 ± 0.55 (0.93–2.77)		
Chicks	New	2007	12	1.12 ± 0.16 (0.83–1.50) a		
Macaroni penguin						
Adults	New	2007	12	2.24 ± 0.29 (1.87–2.75) A	−20.07 ± 0.81 B	9.96 ± 0.36 B
Adults	Old	2006	12	2.08 ± 0.35 (1.64–2.90)		
Chicks	New	2007	12	0.36 ± 0.07 (0.25–0.52) b		
Southern rockhopper penguin						
Adults	New	2007	12	1.96 ± 0.41 (1.22–2.62) A	−19.90 ± 0.41 B	9.96 ± 0.35 B
Adults	Old	2006	12	1.92 ± 0.35 (1.30–2.49)		
Chicks	New	2007	12	0.27 ± 0.06 (0.20–0.37) c		
Gentoo penguin						
Estacade						
Adults	New	2007	12	5.85 ± 3.00 (1.28–9.43) B	−16.46 ± 1.64 C	13.12 ± 1.60 C
Adults	Old	2006	12	4.96 ± 2.44 (2.36–8.74)		
Chicks	New	2007	12	2.45 ± 0.67 (1.14–3.66) d		
Penn Island						
Adults	New	2007	12	1.44 ± 0.44 (0.77–2.06)		

[a] Groups with the same letter are not statistically different (pairwise Wilcoxon comparisons with Bonferroni correction, $p < 0.05$). Upper and lower-case letters are for adults and chicks, respectively.

Models including feather $\delta^{15}N$ values as covariate presented a better fit to the data than those including feather $\delta^{13}C$ values. Two models, Log Hg = $\delta^{15}N$ and Log Hg = $\delta^{15}N$ + species, had a similar level of support in the data, the most accurate being Log Hg = $\delta^{15}N$ according to the principle of parsimony.

3.2. Influence of year and age-class on feather Hg concentrations

Feather Hg concentrations in adult penguins were not significantly different between the two consecutive years, with every species exhibiting similar levels in 2006 and 2007 (Fig. 1). Interestingly, feather Hg concentrations were highly and positively correlated between the two years at the individual level (pooled data from the four species) (Pearson correlation, $r = 0.89$, $p < 0.0001$, $n = 48$, Fig. 2).

Chicks showed significantly lower feather Hg concentrations than adults for all the four species (Fig. 1). Moreover, KP chicks were significantly less contaminated than immature birds (pairwise Wilcoxon comparisons with Bonferroni correction, $p = 0.005$, $n = 36$; Table 2), while feather Hg concentrations of immature and mature birds were not significantly different (pairwise Wilcoxon comparisons with Bonferroni correction, $p = 0.220$, $n = 36$; Table 2). Finally, chick feather Hg concentrations differed significantly between species (Kruskal–Wallis, $H = 41.16$, $p < 0.0001$, $n = 48$), in the decreasing order GP > KP > MP > SRP (Table 2).

Table 3
AIC_c model ranking for adult feather Hg concentrations within the Kerguelen penguin community. Abbreviations: AIC_c, Akaike's Information Criteria adjusted for small sample-size values; w_i, AIC_c weights; R^2adj, R-squared adjusted.

Models	No. of parameters	AIC_c	ΔAIC_c[a]	w_i[b]	R^2adj
$\delta^{15}N$	3	26.30	0.00	0.54	0.67
Species + $\delta^{15}N$	6	26.68	0.38	0.44	0.69
Species + $\delta^{15}N$ + species:$\delta^{15}N$	9	32.72	6.41	0.02	0.69
Species + $\delta^{13}C$ + species:$\delta^{13}C$	9	40.08	13.78	0.00	0.64
Species + $\delta^{13}C$	6	40.10	13.79	0.00	0.59
$\delta^{13}C$	3	41.24	14.94	0.00	0.55
Species	5	49.20	22.89	0.00	0.49
Null	2	77.85	51.54	0.00	0.00

[a] Scaled ΔAIC_c; $\Delta AIC_c = 0.00$ is interpreted as the best fit to the data among the models.
[b] Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

3.3. Spatial and individual variation of feather Hg concentrations: the case study of adult GP

In 2007, feather Hg concentrations of adult GP differed according to the colony of origin, with individuals breeding in an enclosed bay (Penn Island) being significantly less contaminated than those breeding at an open-sea location (Estacade) (Wilcoxon, $W = 137$, $p < 0.0001$, $n = 24$). Unlike Penn GP, Estacade birds showed a high variance in their feather Hg concentrations, and also in their $\delta^{13}C$ and $\delta^{15}N$ values (Table 2). Interestingly, feather Hg concentrations from Estacade penguins were significantly and positively related to both their $\delta^{13}C$ and $\delta^{15}N$ values (Pearson correlation, $r = 0.61$, $p = 0.037$ and $r = 0.74$, $p = 0.006$, respectively, $n = 12$) (Fig. 3).

4. Discussion

4.1. Adult Hg levels: comparison with other seabirds and other areas

The Kerguelen penguin community presented two groups of Hg contamination: on the one hand KP, MP and SRP with similar feather Hg concentrations and on the other hand GP, with ~3 fold higher levels than the three other species. Based on multifactorial analysis, the effect of species explained a considerable proportion of the variance in feather Hg levels, as previously shown in other subantarctic seabird communities (Anderson et al., 2009; Blévin et al., 2013). Furthermore, the interspecific differences were consistent between years. Indeed, no inter-annual variation was detected in feather Hg concentrations between 2006 and 2007 both at the species and individual levels. This result is consistent with the previous findings of Scheifler et al. (2005) on adult KP from the Crozet Islands (southern Indian Ocean), which showed no variation in feather Hg concentrations between 2000 and 2001.

Overall, feather Hg concentrations of Kerguelen penguins were in the same order of magnitude as those reported by previous studies on penguins, but discrepancies at the species level exist. While feather Hg concentrations of KP from Crozet (Scheifler et al., 2005) were almost identical to those found in this study, GP at South Georgia (southern Atlantic Ocean) presented lower feather Hg (Becker et al., 2002) than their Kerguelen conspecifics. Interestingly, South Georgia GP presented lower Hg concentrations in the feathers than the sympatrically breeding MP, which is opposite to the findings of the present study. These contrasting results within the same species breeding at

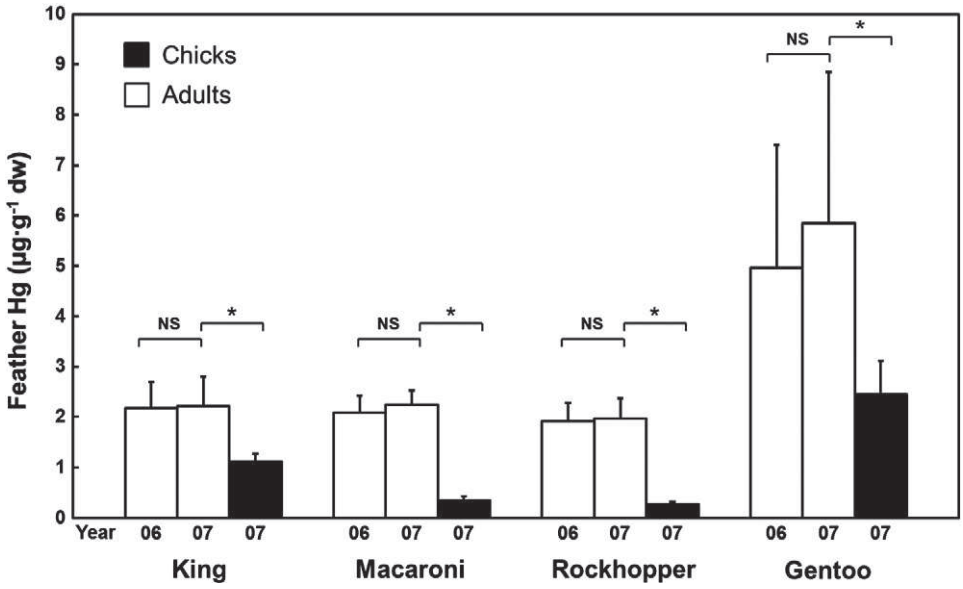

Fig. 1. Feather Hg concentrations (µg g^{-1} dw) of penguin chicks and adults in 2006 and 2007 at Kerguelen Islands. *Statistically different (Wilcoxon, $p < 0.05$); NS: not significant (Wilcoxon test for matched samples, $p > 0.05$). Values are means + SD.

distinct sites are likely related to different feeding habits. Indeed, diet composition of a given species can vary according to the breeding location (e.g., Lescroël et al., 2004; see Section 4.3.), and the diet is the main factor explaining Hg burden in seabirds (e.g., Bocher et al., 2003; Monteiro et al., 1998; Stewart et al., 1999).

Feather Hg concentrations of penguins rank low to intermediate when compared to most procellariiform seabirds that breed in the Southern Ocean. For instance, penguins are usually more contaminated than small zooplankton-eating petrels, but are less contaminated than sympatrically breeding albatrosses and large petrels, which generally present feather levels > 6–10 µg g^{-1} (Anderson et al., 2009; Bargagli et al., 1998; Becker et al., 2002; Lock et al., 1992). Finally, penguins present lower Hg concentrations in the feathers than other diving seabirds from the Southern Ocean, like the South Georgian shag and the common and South Georgian diving petrels (Anderson et al., 2009; Becker et al., 2002). These Hg differences between penguins and other seabirds can be related more to intrinsic (e.g. physiology) and extrinsic factors (e.g. diet composition) than to phylogeny (Anderson et al., 2009; Stewart et al., 1999).

4.2. Chick Hg levels and age-class variation

As expected, feather Hg concentrations of adults were significantly higher than those of chicks in all Kerguelen penguin species. The same result has been reported by several other studies on birds (e.g., Bond and Diamond, 2009; Burger, 1993; Thompson et al., 1991), including penguins (Bargagli et al., 1998), the most likely explanation being that adults have a longer period to bioaccumulate Hg than chicks. Indeed, feather Hg levels in chicks only represent exposure during the chick-rearing period, as the Hg burden inherited from the mother *via* the egg is excreted, at least partially, in the down (Bearhop et al., 2000a; Becker et al., 1993; Stewart et al., 1997) and is highly diluted in the internal tissues during growth (Ackerman et al., 2011). As the length of the chick-rearing period varies among penguins (for review, Williams, 1995), the ratio of adult to chick feather Hg might be expected to be higher in species with shorter chick-rearing periods (Furness et al., 1990). In the present study, the ratios were as high as ~6 and ~7 in MP and SRP, respectively, having chick-rearing periods of ~70 days (for review, Williams, 1995). In contrast, the ratio was only ~2 in KP, because their chick-rearing period is long (~315 days; Williams, 1995) and close to the adult

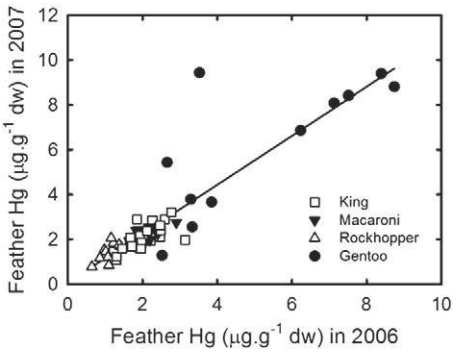

Fig. 2. Positive correlation between feather Hg concentrations (µg g^{-1} dw) of individual adult penguins during two consecutive years (2006 and 2007) at Kerguelen Islands.

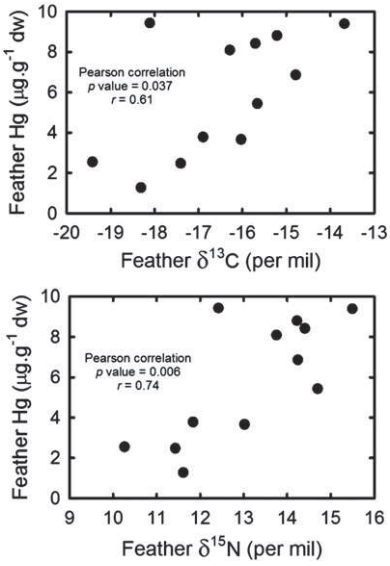

Fig. 3. Positive correlations between new feather Hg concentrations (µg g^{-1} dw) and $\delta^{13}C$ and $\delta^{15}N$ values (‰) of individual adult gentoo penguins from the open-water site (Estacade) at Kerguelen Islands.

inter-moult period (~365 days). Accordingly, feather Hg concentrations of immature KP were similar to those of adults, in agreement with the identical duration of their inter-moult periods. A remarkable exception was the GP with an adult to chick feather Hg ratio of only ~2, even if their chick-rearing period is short (72 days on average; Williams, 1995). The time of exposure is thus not the only determinant of Hg concentrations in the feathers, but other intrinsic or extrinsic factors are likely involved. For instance, the small difference in contamination between the two GP age classes might be linked to the fact that the diet of adults was less Hg-enriched than that of the chicks. Indeed, seabirds can present parent–offspring dietary segregation, as adults tend to provision their chicks with larger and more energy-rich prey than those they capture for self-feeding (Alonso et al., 2012; Dänhardt et al., 2011; Wilson et al., 2004).

Interestingly, interspecific differences were not identical when considering either adults or chicks. With the exception of GP, showing the highest feather Hg concentrations in both age classes, the other species presented feather Hg concentrations in the order KP > MP > SRP in chicks while they were similar in adults. Two non-exclusive factors can explain this result: the length of the chick-rearing period and the chick diet. Indeed, the long chick rearing duration of KP could account for its high chick feather Hg level with respect to MP and SRP. Moreover, the main food items of KP chicks at different locations are mesopelagic fish (e.g., Bost et al., 2002; Cherel et al., 1996), which are known to accumulate high Hg burdens (Bustamante et al., 2003; Chouvelon et al., 2012; Monteiro et al., 1998) in relation to elevated Hg methylation in low oxygen, mesopelagic waters (for review, see Fitzgerald et al., 2007). By contrast, MP and SRP chicks have a mixed diet, relying mostly on swarming pelagic crustaceans (Table 1), which have lower Hg concentrations than fish (e.g., Anderson et al., 2009). This pattern is in agreement with the findings of Blévin et al. (2013) for Hg concentrations in the feathers of Kerguelen seabird chicks, which increased roughly in the order crustacean- < fish- ≤ squid- ≤ seabird-consumers. Their data ranked the penguin species in a low (KP, MP, SRP) to intermediate (GP) position within the seabird community. As penguins are not the most contaminated family among Antarctic and subantarctic seabirds, it could be argued that they are not the best sentinel species of Hg contamination in the Southern Ocean. However, penguins can be regarded as reliable Hg indicators over highly-mobile, flying species when considering their moult strategies and their feeding ecology.

4.3. Hg contamination and penguin food and feeding ecology

4.3.1. Trophic variation of feather Hg concentrations in adult penguins

Stable isotopes segregated the penguin community into three trophic niches: two different niches for KP and GP and an overlapping niche for MP and SRP. As stated above, two groups were discriminated according to the Hg levels: on the one hand KP, MP and SRP and on the other GP. This is in agreement with several studies showing weak or no association between stable isotopes and Hg levels in seabird feathers (Bond and Diamond, 2009; Ramos et al., 2009; Thompson et al., 1998). Indeed, feathers reflect the isotopic composition of the bird's diet at the time of their synthesis (Kelly, 2000), while feather Hg levels reflect dietary exposure during feather growth but also Hg stored in soft tissues during the inter-moult period. Stable isotopes and Hg integration are therefore temporally uncoupled in the feathers of adult birds (Bond and Diamond, 2009; Bond, 2010; Thompson et al., 1998). Nevertheless, individual foraging preferences are maintained over extended periods in penguins, with respect to both foraging area and diet, during and outside the breeding season (Bost et al., 2009; Cherel et al., 2007; Thiebot et al., 2011a, 2011b, 2012). Unlike other seabirds, the relationship between isotopic ratios and Hg concentrations is therefore not spurious in the feathers of adult penguins. In the present study, both $\delta^{13}C$ and $\delta^{15}N$ values were correlated to Hg concentrations in the feathers. With respect to the foraging habitat ($\delta^{13}C$ values), the high Hg concentrations in the feathers of GP, the only inshore feeder, is in agreement with the Me–Hg enrichment of coastal benthic areas (Fitzgerald et al., 2007). Nevertheless, multifactorial analyses revealed that the trophic position ($\delta^{15}N$ values) had a stronger effect on Hg concentrations in the feathers than the foraging habitat. This result verifies our hypothesis stating that Hg concentration increases with trophic level, thus indicating Hg biomagnification in the marine ecosystem exploited by the penguins, i.e. Kerguelen neritic and oceanic waters.

4.3.2. Spatial and individual variation of feather Hg concentrations: the case study of adult GP

While inter-specific differences in seabird Hg contamination have been extensively studied, the causes underlying variation within the same species are less well understood (Bearhop et al., 2000b). However, intra-specific variations in seabird Hg concentrations potentially reflect the effect of interacting intrinsic factors like age, sex and size (Burger, 1993; Monteiro and Furness, 1995) and extrinsic factors, such as foraging habitat and diet (Bearhop et al., 2000a, 2000b; Ramos et al., 2009; Stewart et al., 1997). In this study, the two sampled sub-populations of GP showed highly different levels of Hg contamination, with Estacade GP displaying on average 4 times higher feather Hg concentrations than their Penn Island conspecifics. Such striking differences in feather Hg concentrations within the same species seem difficult to attribute to physiological factors. Instead, Kerguelen GP show a great variability in their trophic ecology depending on the colony location (Bost and Jouventin, 1990; Lescroël and Bost, 2005; Lescroël et al., 2004). For instance, GP from the enclosed bay feed extensively on swarming crustaceans (85% of the diet by mass at different sites of the bay; Lescroël et al., 2004). Accordingly, they presented low Hg concentrations in the feathers, as observed in species relying mainly on pelagic crustaceans (Becker et al., 2002; Bocher et al., 2003; Stewart et al., 1999). On the other hand, Kerguelen GP breeding at the open-sea location (Estacade) present a more diversified diet, including a large proportion of benthic fish, but also pelagic crustaceans (71% and 13% of the overall diet by mass, respectively; Lescroël et al., 2004). Benthic organisms are in close association with the sediment where Hg methylation is high due to low oxygen concentrations and low solar radiation (Fitzgerald et al., 2007). They consequently tend to accumulate high levels of Hg (Bustamante et al., 2006; Storelli et al., 2005, 2007), most probably explaining the considerable Hg burden of Estacade GP. Furthermore, fish display higher Hg concentrations than crustaceans (Chouvelon et al., 2012) and virtually all the Hg (i.e. >95%) in fish is methylated (Bloom, 1992) and is therefore bioavailable for upper trophic levels. The different Hg contamination of the two colonies seems therefore to rely mainly on diet preferences.

The pattern of Hg contamination in the two colonies differed also when considering inter-individual variation. Indeed, Penn and Estacade GP showed a small and a large variance in feather Hg concentrations, respectively. Moreover, there was an overlap in feather Hg concentrations between the two colonies, with some individuals of Estacade GP showing similar levels of Penn GP and then a continuous increase of the Hg concentration. Importantly, feather Hg concentrations of Estacade GP were significantly and positively correlated to stable isotopes (Fig. 3). This indicates a succession of specialised foraging individuals among Estacade GP, ranging from birds feeding almost exclusively on pelagic crustaceans (reflected by low feather $\delta^{13}C$ and $\delta^{15}N$ values) to birds feeding almost exclusively on benthic fish (high feather $\delta^{13}C$ and $\delta^{15}N$ values), therefore showing low to high feather Hg concentrations. Individual dietary specialisation is thus crucial in establishing Hg contamination in this species.

5. Conclusions

This work provides new insights into the relationship between seabird trophic ecology and Hg burdens, emphasising the role of

species and individual foraging specialisation in shaping feather Hg levels. Individual specialisation of foraging strategies is believed to be conserved over long periods in seabirds (Bearhop et al., 2006; Ceia et al., 2012) and could therefore put some individuals at a risk of high Hg contamination levels over the long-term, as illustrated by the positive inter-annual correlation in feather Hg concentrations. Although it is difficult to link observed Hg tissue concentrations to negative effects in natural bird populations (Burger and Gochfeld, 2004), 50% of adult Estacade GP exceeded the commonest used feather toxicity threshold of 5 μg g^{-1} dw (i.e. Burger and Gochfeld, 1997). This sub-population of GP could therefore serve as a model to investigate adverse effects of Hg contamination on seabirds in the wild. On the other hand, penguin species showing low inter-individual variation in Hg levels could be used as efficient indicators of Hg contamination. Considering their abundance, distribution and highly specialised diet (mesopelagic fish), KP in particular could be useful as a bioindicator species of Hg bioavailability in the Southern Ocean. A comparison of the results of this study with those obtained from other penguin populations which breed at different subantarctic and Antarctic sites could help to depict the geographical distribution of Hg contamination in the Southern Ocean. Furthermore, penguins could be useful as monitors of temporal trends of global Hg contamination, as their foraging strategies are maintained over the long-term. A change in Hg bioavailability in the food webs of the Kerguelen Islands, which are far-remote from direct anthropogenic inputs, would indeed indicate a change of Hg fluxes on a much wider spatial scale.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Paper 3

Mercury exposure in a large subantarctic avian community

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Mercury exposure in a large subantarctic avian community

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ABSTRACT

Mercury (Hg) contamination poses potential threats to ecosystems worldwide. In order to study Hg bioavailability in the poorly documented southern Indian Ocean, Hg exposure was investigated in the large avian community of Kerguelen Islands. Adults of 27 species (480 individuals) showed a wide range of feather Hg concentrations, from 0.4 ± 0.1 to 16.6 ± 3.8 μg g^{-1} dry weight in Wilson's storm petrels and wandering albatrosses, respectively. Hg concentrations increased roughly in the order crustacean- < fish- ≤ squid- ≤ carrion-consumers, confirming that diet, rather than taxonomy, is an important driver of avian Hg exposure. Adults presented higher Hg concentrations than chicks, due to a longer duration of exposure, with the only exception being the subantarctic skua, likely because of feeding habits' differences of the two age-classes in this species. High Hg concentrations were reported for three species of the poorly known gadfly petrels, which merit further investigation.

1. Introduction

Mercury (Hg) is a pervasive non-essential metal affecting ecosystem health. Despite its natural origin, Hg has been mobilized by human activities such as mining and fossil-fuel combustion (UNEP, 2013), thus resulting in a significant increase in Hg available for cycling among land, air and the ocean since pre-industrial times (Selin, 2009). Hg emissions are transported through the atmosphere on a hemispheric-to-global scale, allowing for transport to remote locations such as sub-polar and polar regions (Fitzgerald et al., 1998). After atmospheric deposition and through biotic and abiotic mechanisms, Hg is readily transformed in methyl-Hg, the highly toxic form that bioaccumulates in the tissues of living organisms and biomagnifies up food webs, especially in aquatic environments (Fitzgerald et al., 2007). Top predators are thus exposed to significant quantities of Hg *via* their diet, providing information on Hg bioavailability within their food webs (Morel et al., 1998). Among consumers, birds have varied levels of ecological, spatial and temporal integration of contaminants depending on species, and they have been identified as effective indicators of Hg bioavailability in both terrestrial and marine environments (Burger and Gochfeld, 2004; Solonen and Lodenius, 1990).

The Kerguelen Islands are a remote subantarctic archipelago in the southern Indian Ocean, where the level of Hg bioavailability is poorly documented (Bocher et al., 2003; Bustamante et al., 2003; Cipro et al., 2014; Cossa et al., 2011). This archipelago hosts a large and highly diverse avian assemblage (35 different breeding species). The community includes a few terrestrial species and many seabirds, with Sphenisciformes (penguins) and Procellariiformes (albatrosses and petrels) dominating by mass and numbers, respectively (Guinet et al., 1996; Weimerskirch et al., 1989). Overall Kerguelen seabirds feed on a few key species of marine organisms, including some crustaceans (euphausiids, hyperiids), fish (myctophids, notothenioids) and cephalopods (oceanic squids) (Bocher et al., 2001; Cherel et al., 2010; Cherel and Hobson, 2005; Guinet et al., 1996), with some seabirds relying extensively on carrion. This biological richness can be related to the large and productive shelf surrounding the archipelago (Blain et al., 2001). Kerguelen seabirds show a wide range of contrasted feeding strategies, with species foraging in the benthic and pelagic environments and ranging from neritic to oceanic waters. Noticeably, the oceanic species forage over a large latitudinal gradient, from subtropical to Antarctic waters (Supplementary Table S1). Kerguelen seabirds therefore offer a unique opportunity to study Hg bioavailability over diverse water masses of the Southern Ocean.

The present study aims to assess Hg bioavailability in the southern Indian Ocean by using birds from the Kerguelen Islands as bioindicators. Hg exposure was evaluated by using body feathers,

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because feathers are the main route of Hg excretion in birds (Braune and Gaskin, 1987). Importantly, this work complements a recent investigation on Hg in chicks (Blévin et al., 2013) by focussing on breeding adults and by including more species. While Hg concentrations in chick feathers are representative of a well-defined, relatively short period of exposure (the chick-rearing period), adult feathers provide a wider perspective on Hg exposure of the species over their whole life cycles (Evers et al., 2005). Thus adult feather Hg concentrations were determined in 27 representative species, including the only two terrestrial birds of the assemblage, in order to: (i) describe Hg exposure in a large number of sympatric bird species from the poorly documented southern Indian Ocean; (ii) compare the exposure pattern to that of avian communities from other subantarctic and oceanic remote locations worldwide; (iii) test the effect of age-class on feather Hg concentrations by using the recently published Hg data on chicks (Blévin et al., 2013), and (iv) investigate the influence of various factors (taxonomy, diet, feeding habitats, moulting patterns) on Hg exposure. Taxonomy, which was not tested in Blévin et al. (2013), was expected to play a minor role in explaining feather Hg concentrations when compared to feeding strategies, since diet is considered to be the main factor driving Hg variation in birds (Becker et al., 2002; Blévin et al., 2013; Bocher et al., 2003; Monteiro et al., 1998; Stewart et al., 1999). In addition, adult birds were expected to show higher feather Hg concentrations than chicks, as they are exposed over a longer period to Hg *via* their diet (Catry et al., 2008; Stewart et al., 1997).

2. Materials and methods

Fieldwork was carried out from 2003 to 2011 on the Kerguelen Islands (49°21′ S, 70°18′ E, Fig. 1), which are located in the southern part of the Polar Frontal Zone, in the immediate vicinity of the Polar Front (Orsi et al., 1995; Park and Gamberoni, 1997). Breeding adults from 27 bird species belonging to 5 orders and 10 families were sampled ($n = 5$ to 33 individuals per species, Supplementary Table S2). Sampling was conducted at different locations of the archipelago, depending on the species breeding sites. Resident neritic seabird species (Kerguelen shag and gentoo penguin) were sampled at colonies close to the open sea, while terrestrial species (lesser sheathbill and Kerguelen pintail) were sampled on islands of the large Morbihan Bay (closed sea). Birds were non-destructively captured by mist net or by hand, depending on species, and released immediately after sampling. A few whole body feathers (6–10) were pulled out from the lower back of the birds and then stored dry in sealed plastic bags until analysis at the University of La Rochelle, France.

Depending on bird and hence feather size, 1 to 5 whole feathers per individual were cleaned, oven-dried to a constant mass and homogenised as described in Blévin et al. (2013). An Advanced Mercury Analyzer spectrophotometer (Alted AMA 254) was used to measure total Hg, which approximates the amount of methyl-Hg in feathers (Bond and Diamond, 2009; Thompson and Furness, 1989a). For each individual, analyses were run in duplicate-triplicate by taking sub-samples of the homogenised feathers (relative standard deviation <10% for each individual). Accuracy was checked using certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 μg g^{-1} dry weight). Our measured values were 0.24 ± 0.01 μg g^{-1} dry weight, $n = 22$. Blanks were analysed at the beginning of each set of samples and the detection limit of the method was 0.005 μg g^{-1} dry weight. Hg concentrations are presented μg g^{-1} dry weight (dw).

Statistical analyses were performed using R 2.15.1 (R Core Team, 2012). Data exploration was performed mainly following Zuur et al. (2010). The influence of taxa (species, genus, family and order) on adult feather Hg concentrations was tested by using generalized linear models (GLM) with a gamma distribution and an inverse link function. Model selection was based on Akaïke's Information Criteria adjusted for small sample sizes (AICc) (Burnham and Anderson, 2002). The sampling year was not included in the models because most species were sampled in only one year (Supplementary Table S2) and thus the year effect would be confounded by the species effect. Nonetheless, no inter-annual differences in feather Hg concentrations were found on the six species that were sampled in two different years (light-mantled sooty albatross, soft-plumaged and Kerguelen petrels, black-bellied storm petrel, South Georgia diving petrel and lesser sheathbill, data not shown). As biometric measurements were not performed on individual birds during the sampling procedure, the effect of size and mass on feather Hg concentrations could not be incorporated in the models. However, mean values of size and mass were obtained for each species from the literature (Supplementary Table S1) and their correlations with mean feather Hg concentrations were tested. Finally, the effect of age-class on feather Hg concentrations was investigated on 21 out of the 27 species by comparing adult data from the present study with chick data from the same Kerguelen locations (Blévin et al., 2013). A significance level of $\alpha < 0.05$ was used for all statistical tests. Results are means ± SD.

General information on the feeding ecology of Kerguelen birds was based on published and unpublished data obtained using various methods (stomach content and stable isotope analyses and tracking devices), and is summarized in Supplementary Table S1. Importantly, dietary information was restricted to the chick food, collected during the chick-rearing period, because parent birds carry significant amounts of food in their stomach at that time only. By contrast, adult diet is poorly known both during and outside the breeding period. The relationship between Hg exposure and trophic ecology was not studied here using stable isotopes, because of the uncoupled temporal integration of Hg and stable isotopes in feathers of adult birds (Bond, 2010; Thompson et al., 1998).

3. Results

Feather Hg concentrations were measured in a total of 480 adult birds from the Kerguelen Islands (details in Supplementary Table S2). Feather Hg concentrations varied widely within the avian community, with means ranging from 0.42 ± 0.13 to 16.6 ± 3.8 μg g^{-1} dw in Wilson's storm petrels and wandering albatrosses, respectively (Fig. 2). The lowest feather Hg concentration occurred in a South-Georgian diving petrel and the highest in a northern giant petrel (0.10 and 32.1 μg g^{-1}, respectively). Model selection showed that species was the most important factor explaining feather Hg concentrations when compared to other taxonomic levels (Table 1). Coefficients of variations (CV) also varied considerably between species, ranging from 13 to 109% (Supplementary Table S2). Mean feather Hg concentration was significantly related to species size (Pearson correlation, $r = 0.50$, $p = 0.008$, $n = 27$), but not to species mass ($r = 0.23$, $p = 0.256$, $n = 27$).

By combining feather Hg data of adults from this study with those of chicks from Blévin et al. (2013), a total of 654 individuals from 21 seabird species were analysed. The model including

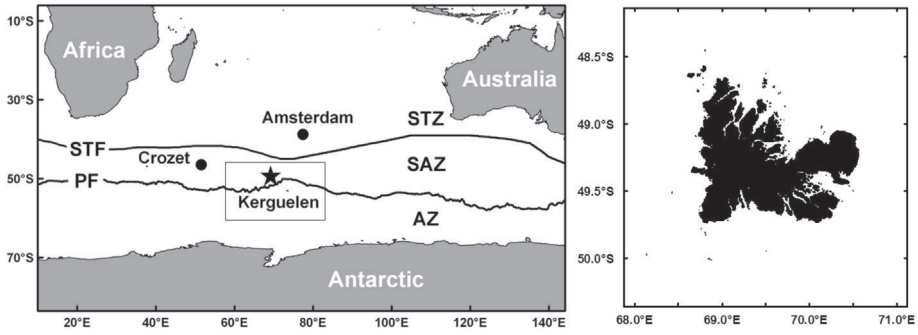

Fig. 1. Map and location of the Kerguelen Islands and of the main oceanic fronts and zones within the southern Indian Ocean. Abbreviations: STF, Subtropical Front; PF, Polar Front; STZ, Subtropical Zone; SAZ, Subantarctic Zone; AZ, Antarctic Zone.

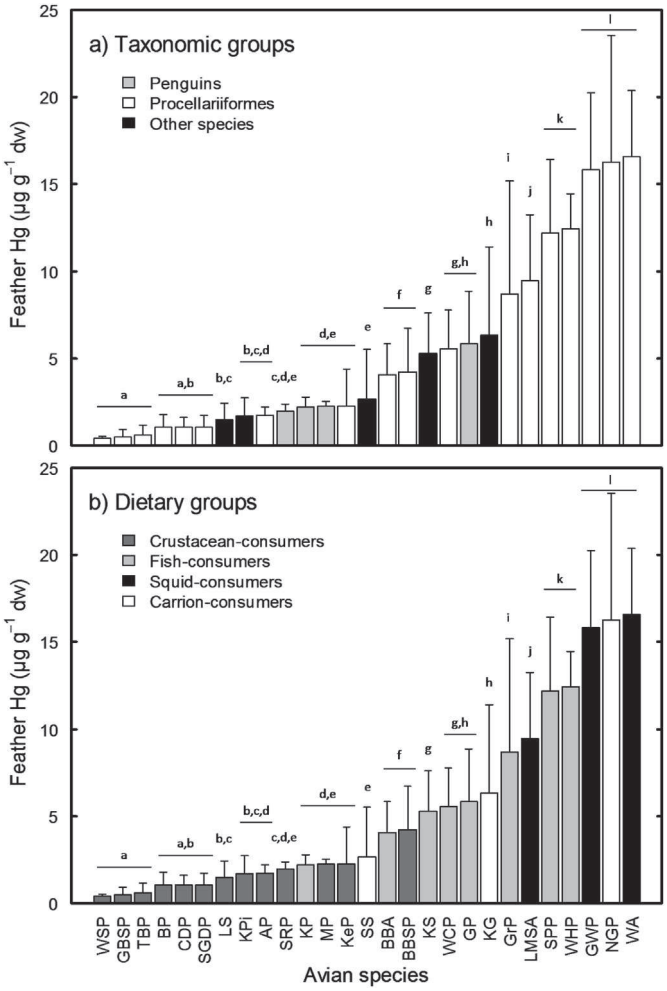

Fig. 2. Inter-specific differences in adult feather Hg concentrations within the Kerguelen avian community. Species are presented according to a) taxonomic groups: penguins (grey), procellariiform seabirds (white) and other species (black); and b) dietary groups: terrestrial species and crustacean- (dark grey), fish- (grey), squid- (black) and carrion- (white) consumers. Species sharing the same letter are not statistically different (Tukey HSD, $p < 0.05$). Values are means ± SD. Species abbreviations: WSP, Wilson's storm petrel; GBSP, grey-backed storm petrel; TBP, thin-billed prion; BP, blue petrel; CDP, common diving petrel; SGDP, South Georgian diving petrel; LS, lesser sheathbill; KPi, Kerguelen pintail; AP, Antarctic prion; SRP, southern rockhopper penguin; KP, king penguin; MP, macaroni penguin; KeP, Kerguelen petrel; SS, subantarctic skua; BBA, black-browed albatross; BBSP, black-bellied storm petrel; KS, Kerguelen shag; WCP, white-chinned petrel; GP, gentoo penguin; KG, kelp gull; GrP, grey petrel; LMSA, light-mantled sooty albatross; SPP, soft-plumaged petrel; WHP, white-headed petrel; GWP, great-winged-petrel; NGP, northern giant petrel; WA, wandering albatross.

species, age-class and their interaction as explaining factors of feather Hg concentrations showed the best fit to the data (Table 1). Feather Hg concentrations were significantly higher in adults than in chicks in all but two species: the blue petrel (no statistical difference) and subantarctic skua (higher chick level) (Fig. 3). The ratio of adult to chick feather Hg concentrations varied between species, ranging from 0.5 to 20.7 in the subantarctic skua and South-Georgian diving petrel, respectively (Supplementary Table S2).

4. Discussion

To the best of our knowledge, this study is the first to report feather Hg concentrations for such a large number of adult sympatric birds, including representative species of all the four families of the order Procellariiformes, namely albatrosses, petrels, storm

Table 1
AIC_c model ranking for adult feather Hg concentrations within the Kerguelen avian community. Models are GLM with a gamma distribution and an inverse link function. Abbreviations: AIC_c, Akaike's Information Criteria adjusted for small sample-sizes values; w_i, AIC_c weights.

Models	N° parameters	AIC_c	ΔAIC_c[a]	w_i[b]
Adults ($N = 480$)				
Species	28	1698	0	1
Genus	22	1719	20	0
Family	11	2226	528	0
Order	6	2422	723	0
Null	2	2459	760	0
Adults and chicks ($N = 654$)				
Species * Age-class	43	1684	0	1
Species + Age-class	23	2276	592	0
Species	22	2471	787	0
Age-class	3	2915	1231	0
Null	2	3037	1353	0

[a] Scaled ΔAIC_c; $\Delta AIC_c = 0.00$ is interpreted as the best fit to the data among the models.
[b] Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

petrels and diving petrels. Hg exposure varied widely within the community, showing a remarkable 40-fold difference between the species with the lowest and highest Hg concentrations. The community exposure pattern agrees with results on chicks (Blévin et al., 2013): small petrels, penguins and terrestrial species generally showed low levels of exposure (<2.5 µg g^{-1} dw), coastal seabirds, *Procellaria* petrels and small albatrosses had intermediate concentrations (<10 µg g^{-1} dw), while *Pterodroma* petrels, northern giant petrels and wandering albatrosses were the species having the highest concentrations (>10 µg g^{-1} dw).

4.1. Comparisons with other avian communities

The pattern of Hg concentrations among members of the Kerguelen avian community fits well with that of other subantarctic

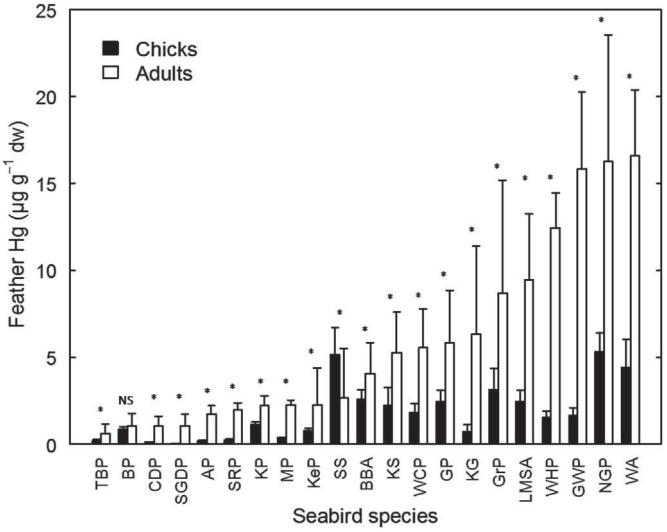

Fig. 3. Age-class differences in feather Hg concentrations within the Kerguelen seabird community. * Statistically different (Wilcoxon, $p < 0.05$); NS: not significant. Values are means ± SD. Species abbreviations: TBP, thin-billed prion; BP, blue petrel; CDP, common diving petrel; SGDP, South Georgian diving petrel; AP, Antarctic prion; SRP, southern rockhopper penguin; KP, king penguin; MP, macaroni penguin; KeP, Kerguelen petrel; SS, subantarctic skua; BBA, black-browed albatross; KS, Kerguelen shag; WCP, white-chinned petrel; GP, gentoo penguin; KG, kelp gull; GrP, grey petrel; LMSA, light-mantled sooty albatross; WHP, white-headed petrel; GWP, great-winged-petrel; NGP, northern giant petrel; WA, wandering albatross.

sites, such as South Georgia, southern Atlantic Ocean (Fig. 4). Feather Hg concentrations were comparable in seabirds from the two localities, with the exception of the northern giant petrel and wandering albatross (Anderson et al., 2009; Becker et al., 2002). This could be related to inter-site dietary differences of these two top predators. Moreover, similar feather Hg concentrations were reported for some species of albatrosses and petrels from the southern Pacific Ocean (Thompson et al., 1990, 1993). Hg bioavailability thus seems to be similar within the three sectors of the Southern Ocean, which agrees well with its circumpolar annular oceanographic structure (Sokolov and Rintoul, 2007). At lower latitudes of the Southern Hemisphere, only the tropical seabird communities from the western Indian Ocean have been investigated, revealing much lower feather Hg concentrations (~0.05–1.5 μg g^{-1}; Catry et al., 2008; Kojadinovic et al., 2007) than in Kerguelen birds. This trend could result from differences in the physical and biological factors driving methyl-Hg production and food web transfer in the two regions (i.e., the atmospheric deposition of inorganic Hg, the rate of primary productivity, the abundance of sinking organic matter and the structure of the microbial community, Mason et al., 2012). The wide range of Hg exposure of Kerguelen birds compares well with avian communities of remote archipelagos of the Northern Hemisphere, namely the tropical Midway Atoll, North Pacific Ocean and the subtropical Azores Islands, North Atlantic Ocean (both ~ 1–20 μg g^{-1}; Burger and Gochfeld, 2000a; Gochfeld et al., 1999; Monteiro et al., 1998). These wide ranges of contamination are related to the presence of highly contaminated species of albatrosses and gadfly petrels within the communities (see below). Indeed, the avian assemblage of the temperate Machias Seal Island, North Atlantic Ocean, which includes neither albatrosses nor gadfly petrels, had lower feather Hg concentrations (0.7–7 μg g^{-1}; Bond and Diamond, 2009). Therefore, the specific composition of the avian communities rather than the proximity to highly industrialized countries seems to be a key factor driving the level of Hg exposure within avian assemblages from open sea regions. In this context, the range of Hg exposure within the Kerguelen avian community is remarkable, as it encompasses the concentrations reported worldwide in remote oceanic locations (Blévin et al., 2013).

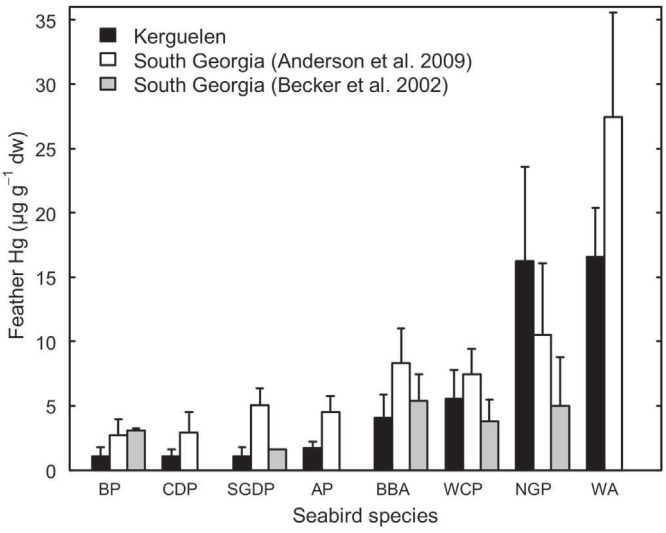

Fig. 4. Comparison of feather Hg concentrations between the same seabird species breeding in the southern Indian Ocean (Kerguelen Islands, present study) and in the southern Atlantic Ocean (South Georgia, Anderson et al., 2009, Becker et al., 2002). Species abbreviations: BP, blue petrel; CDP, common diving petrel; SGDP, South Georgian diving petrel; AP, Antarctic prion; BBA, black-browed albatross; WCP, white-chinned petrel; NGP, northern giant petrel; WA, wandering albatross.

4.2. Influence of taxonomy

The best taxonomic explanatory variable of feather Hg concentrations in the Kerguelen community was species, as it integrates a large range of ecological, behavioural, physiological and life-history traits that are susceptible to drive variation in feather Hg concentrations (Anderson et al., 2009; Bond and Diamond, 2009). Although inter-specific differences in avian Hg exposure have often been investigated, taxonomic-related variations were rarely tested in a large number of species (Anderson et al., 2009; Ochoa-Acuna et al., 2002). Here, statistical models including genus, family or order as explanatory variables had a poor fit to feather Hg data. Indeed, closely-related species at Kerguelen often showed very different levels of exposure. For example, black-bellied storm petrels displayed higher Hg concentrations than the other two Hydrobatidae species, despite similar size and life-history traits. The same pattern was highlighted for Sphenisciformes, with the gentoo penguins having higher feather Hg concentrations than the other three penguin species (Carravieri et al., 2013). Therefore, the effect of taxonomy seems to play a minor role in avian Hg exposure when compared to other ecological factors (Becker et al., 2002; Lock et al., 1992; Stewart et al., 1999). Nevertheless, the present study provides new and interesting results regarding a particular taxonomic group: the gadfly petrels (genus *Pterodoma*, Warham, 1990), which were amongst the species with the highest Hg concentrations (Fig. 2). Our data together with a review of the scientific literature (Table 2) point out the high Hg exposure of almost all the *Pterodroma* petrels so far investigated, including species living in different marine ecosystems. Nevertheless, the Barau's Petrel *P. baraui* from La Réunion Island, western Indian Ocean, showed surprisingly low feather Hg concentrations (1.0 μg g^{-1}), suggesting again a regional trend of low Hg bioavailability at tropical latitudes of the Indian Ocean (see Subsection 4.1).

4.3. Influence of diet and feeding habitat

Inter-specific variability in Hg exposure is typically attributed to diet (Arcos et al., 2002; Monteiro et al., 1998; Stewart et al., 1999). Since Hg is efficiently biomagnified up food webs (Atwell et al., 1998; Campbell et al., 2005; Jarman et al., 1996), high trophic level prey, such as fish and cephalopods show higher Hg concentrations than crustaceans and other planktonic organisms (Bustamante et al., 2006; Kojadinovic et al., 2006; Stewart et al., 1997). This is consistent with feather Hg concentrations of Kerguelen birds increasing roughly in the order crustacean- $<$ fish- $\leq$ squid- $\leq$ carrion-consumers (Fig. 2b), as previously shown in chicks (Blévin et al., 2013). This confirms that Hg is efficiently biomagnified and that diet plays a key role in explaining Hg exposure. Accordingly, the positive correlation highlighted between bird size and feather Hg concentrations is likely explained by a trophic effect, as larger avian species tend to occupy higher trophic positions and/or to consume larger prey items (Burger and Gochfeld, 2000a).

Differences in dietary exposure over diverse habitats within and outside the breeding period can also account for important variability in feather Hg concentrations (Anderson et al., 2009; Blévin et al., 2013). Here, inshore non-migratory species (Kerguelen shag, kelp gull, gentoo penguin), which feed on benthic organisms, showed intermediate to high Hg concentrations. This agrees with high Hg bioavailability in benthic environments due to methyl-Hg production in coastal marine sediments (Bustamante et al., 2006; Fitzgerald et al., 2007). In the oceanic domain, methyl-Hg concentration reaches a maximum in mesopelagic waters (Driscoll et al., 2013; Fitzgerald et al., 2007), resulting in enhanced contamination of mesopelagic prey (Chouvelon et al., 2012, Choy et al., 2009).

Table 2
An overall synthesis of Hg concentrations in body feathers of adult gadfly petrels. Values are means ± SD with ranges in parentheses.

Species	Site	Ocean	Breeding region	*n*	Hg (μg g^{-1} dw)	Reference
Atlantic petrel (*Pterodroma incerta*)	Gough Island	South Atlantic	Subantarctic	23	13.9 ± 3.6[a]	Thompson et al. (1990)
	Gough Island	South Atlantic	Subantarctic	15	13.5 ± 4.1 (3.9– 20.1)[a]	Thompson et al. (1993)
Barau's petrel (*Pterodroma baraui*)	La Réunion Island	Indian	Tropical	20	1.0 ± 0.3	Kojadinovic et al. (2007)
Bonin petrel (*Pterodroma hypoleuca*)	Midway Atoll	North Pacific	Subtropical	27	19.7 ± 1.1[b]	Gochfeld et al. (1999), Burger and Gochfeld (2000a)
Great-winged petrel (*Pterodroma macroptera*)	Kerguelen Archipelago	South Indian	Subantarctic	14	15.8 ± 4.4 (9.8–27.1)	This study
Juan Fernandez petrel (*Pterodroma externa*)	Juan Fernandez Archipelago	South Pacific	Subtropical	5 (M)	4.2 ± 0.3	Ochoa-Acuna et al. (2002)
				11 (F)	3.9 ± 0.2	
Soft-plumaged petrel (*Pterodroma mollis*)	Gough Island	South Atlantic	Subantarctic	21	10.3 ± 2.3[a]	Thompson et al. (1990)
	Gough Island	South Atlantic	Subantarctic	17	9.8 ± 2.3 (5.4–13.4)[a]	Thompson et al. (1993)
	Kerguelen Archipelago	South Indian	Subantarctic	19	12.2 ± 4.2 (4.7–25.5)	This study
White-headed petrel (*Pterodroma lessonii*)	Kerguelen Archipelago	South Indian	Subantarctic	10	12.4 ± 2.0 (9.2–17.1)	This study

Studies with too low numbers of sampled individuals ($n < 4$) were excluded.
[a] Values are in μg g^{-1} wet weight.
[b] Values are means ± SE.

This could explain the high feather Hg concentrations of oceanic species relying extensively on mesopelagic fish and cephalopods (e.g. gadfly petrels, Ochoa-Acuna et al., 2002, Ridoux, 1994). On the other hand, migratory seabirds can travel thousands of kilometres away from their breeding sites after reproduction (e.g., Warham, 1990), being potentially exposed to different quantities of Hg. Here, species visiting northern subtropical and neritic waters during the non-breeding period (e.g. the wandering albatross) tended to have higher feather Hg concentrations than those that forage predominantly within the limits of the Southern Ocean year-round (e.g. the light-mantled sooty albatross) (Cherel et al., 2013). However, further investigations on i) the poorly known feeding strategies outside the breeding season and ii) Hg distribution and speciation in the Southern Ocean could elucidate the rationale of this latitudinal trend (see Blévin et al., 2013).

4.4. Influence of moulting patterns

Feather Hg concentrations reflect blood Hg levels at the time of moult (Bearhop et al., 2000; Evers et al., 2008). This means dietary Hg but also Hg accumulated over the inter-moult period and remobilized during feather synthesis (Furness et al., 1986; Thompson et al., 1998). Another important intrinsic driver of Hg concentrations in feathers is therefore the timing, duration and frequency of moult. For instance, the irregular and infrequent moulting patterns of large albatrosses are believed to contribute significantly to high Hg concentrations in their feathers (Anderson et al., 2009; Becker et al., 2002). However, enhanced feather Hg concentrations were also reported in Kerguelen species with annual moult cycles, like great-winged and white-chinned petrels. This indicates that moulting patterns alone cannot explain all the inter-specific variation in Hg concentrations. In Procellariiformes, demethylation of Hg in the liver appears to be a significant detoxification strategy (Thompson and Furness, 1989b; Thompson et al., 1993). The efficiency of demethylation mechanisms is species-dependent and could contribute to explain the inter-specific differences in feather Hg concentrations among members of the Kerguelen avian community.

4.5. Adults and chicks

As previously shown by several other studies (e.g., Burger and Gochfeld, 2000b; Catry et al., 2008), feather Hg concentrations were higher in adults than in chicks in almost all Kerguelen species (Fig. 3). Indeed, adults have more time to bioaccumulate Hg in their tissues during the long inter-moult period (≈one year) before excreting it in feathers (Monteiro et al., 1995; Thompson et al., 1991). By contrast, chick feather Hg concentrations represent the dietary exposure over the chick-rearing period (Ackerman et al., 2011; Becker et al., 1993), which ranges from several weeks to several months in Kerguelen species. Assuming a similar rate of Hg intake of adults and chicks, species with short and long chick-rearing periods should show high and low adult-to-chick ratios in feather Hg concentrations, respectively. The hypothesis was verified in the two diving petrels (high ratios, ≥9) and in the wandering albatross and northern giant petrel (low ratios, ~ 2–4), respectively. Exceptions to this pattern are likely indicative of differential relative Hg exposures in the two age-classes, as observed in blue petrels and subantarctic skuas (ratios: 1.3 and 0.5, respectively). Adult blue petrels from the Kerguelen Islands feed at the same low trophic level in cold Antarctic waters both during the breeding and moulting periods (Cherel et al., 2002, 2006). By contrast, chick food includes a significant proportion of mesopelagic fish (Cherel et al., 2002; Connan et al., 2008), which is consistent with their enhanced Hg exposure over adults. At Kerguelen, subantarctic skua chicks are mainly fed with blue petrels (Mougeot et al., 1998), thus explaining their high feather Hg concentrations. The chick data are in accordance with previous results on chicks of the closely-related great skua that feeds on bird meat (Stewart et al., 1997). The low Hg concentrations of adult subantarctic skuas are puzzling and strongly suggest that moulting adults do not rely on small petrels for feeding. Indeed, previous findings on subantarctic skuas from South Georgia suggest that they have a mixed diet of zooplankton and low trophic-level prey over the wintering grounds (Phillips et al., 2007). Therefore, both the duration of Hg exposure and feeding habits are key factors explaining differences in feather Hg concentrations between seabird chicks and adults.

5. Conclusions

Results from this study reinforce previous findings showing that taxonomy plays a minor role in determining avian Hg exposure when compared to feeding strategies (Stewart et al., 1999). Our results confirm that Hg concentrations are very high in some subantarctic birds, with many species showing levels of potential concern. The most common used toxic threshold of feather Hg concentration in birds is 5 μg g^{-1} (e.g., Evers et al., 2008). Here, this level was exceeded by some individuals of 11 seabird species and by all individuals of wandering albatross, northern giant petrel, and white-headed and great-winged petrels. Although subantarctic

species may have evolved to cope with high Hg exposure in their environment (Blévin et al., 2013; Thompson et al., 1993), there is an urgent need to investigate the inter- and intra-specific physiological differences of Hg metabolism, excretion and toxicity, in order to establish whether some species, or some particular individuals, could be at risk. Evidence of Hg consequences on breeding, hatchling and fledging success has indeed been reported in polar birds (Goutte et al. in press; Tartu et al., 2013). Such investigations on risk related to Hg exposure are particularly relevant in the context of global warming that would favour the methylation rate of Hg in the Ocean (Cossa, 2013). Finally, the present study enables selecting the white-headed petrel as a good bioindicator species of Hg bioavailability in the Southern Ocean, considering its high level of exposure and low intra-specific variation. The white-headed petrel adds to the list of Kerguelen bioindicator species recently identified according to their foraging ecology and exposure patterns, i.e. the gentoo and king penguins, the black-browed, light-mantled sooty and wandering albatrosses (Blévin et al., 2013; Carravieri et al., 2013). The periodic examination of feather Hg concentrations in species from these remote regions over the long-term will make it possible to monitor temporal trends of Hg bioavailability to predators in the open ocean in relation to global trends of Hg emissions.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.03.017.

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Appendix A – Supplementary material

Supplementary Table S1

Species, biometric data, foraging habitats and dietary habits of marine and terrestrial birds breeding at the Kerguelen Islands.

Species	Abb.	Size (cm)[a]	Mass (kg)[a]	Foraging habitat			Chick food	References
				Breeding (horizontal, vertical)	Moulting (non-breeding)	Isotopes (adult feathers)		
Spheniscidae								
King penguin (*Aptenodytes patagonicus*)	KP	90	13.3	oceanic; epi-mesopelagic	unknown (likely oceanic)	Polar Front	mesopelagic fish	Bost el al. (2002), Cherel et al. (2010)
Gentoo penguin (*Pygoscelis papua*)	GP	83	6.5	neritic (open sea); benthic, pelagic	resident all year	Neritic waters	benthic fish (pelagic crustaceans)	Lescroël et Bost (2005)
Macaroni penguin (*Eudyptes chrysolophus*)	MP	71	4.9	neritic, oceanic; epipelagic	Polar Frontal Zone, oceanic	Subantarctic	pelagic crustaceans (fish)	Cherel et al. (2010), Thiebot et al. (2011a,b)
Southern rockhopper penguin (*Eudyptes chrysocome filholi*)	SRP	50	2.9	neritic (closed sea); epipelagic	Subantarctic and Polar Frontal Zone, oceanic	Subantarctic	pelagic crustaceans (fish)	Tremblay and Cherel (2000, 2003); Cherel et al. (2010); Thiebot et al. (2012)
Diomedeidae								
Wandering albatross (*Diomedea exulans*)	WA	123	8.8	oceanic, sea surface	subtropical waters, oceanic	Subtropics	fish and cephalopods	Cherel et al. (2013)
Black-browed albatross (*Thalassarche melanophrys*)	BBA	88	3.8	neritic (open sea); sea surface	subtropical waters (southern Australia), neritic	Subtropics	benthopelagic fish (cephalopods)	Cherel et al. (2000a,b); Cherel et al. (2013)
Light-mantled sooty albatross (*Phoebetria palpebrata*)	LMSA	84	3.1	oceanic; sea surface	subantarctic waters; oceanic	Subantarctic, Antarctic	cephalopods (crustaceans, carrion)	Ridoux (1994)[a]; Cherel et al. (2013)
Procellariidae								
Northern giant petrel (*Macronectes halli*)	NGP	88	4.4	on land and at sea surface	subantarctic to subtropical waters, oceanic	Subantarctic	carrion/seabirds	Ridoux (1994)[a]; Thiers et al. (2014)
Grey petrel (*Procellaria cinerea*)	GrP	50	1.1	oceanic; sea surface	unknown	Subtropical Front	fish (cephalopods)	Ridoux (1994)[a]
White-chinned petrel (*Procellaria aequinoctialis*)	WCP	55	1.3	oceanic; sea surface	northern waters (Benguela current), neritic	Subtropics (Benguela)	fish (cephalopods, crustaceans)	Delord et al. (2010); Péron et al. (2010)
Great-winged petrel (*Pterodroma macroptera*)	GWP	39	0.56	oceanic; sea surface	unknown	Subtropics	cephalopods (crustaceans)	Ridoux (1994)[a]
White-headed petrel (*Pterodroma lessonii*)	WHP	43	0.70	oceanic; sea surface	unknown	Subantarctic	fish (cephalopods)	Zotier (1990)
Soft-plumaged petrel (*Pterodroma mollis*)	SPP	35	0.30	oceanic; sea surface	unknown	Subtropics	fish (cephalopods, crustaceans)	Unpublished data
Kerguelen petrel (*Aphrodroma brevirostris*)	KeP	35	0.36	oceanic; sea surface	Unknown	High Antarctic	crustaceans	Ridoux (1994)[a]

Blue petrel (*Halobaena caerulea*)	BP	29	0.20	oceanic; sea surface	Antarctic waters, oceanic	High Antarctic	crustaceans (mesopelagic fish)	Cherel et al. (2002a); Cherel et al. (2006)
Antarctic prion (*Pachyptila desolata*)	AP	26	0.16	oceanic; sea surface	subtropical waters, oceanic	Subtropics	crustaceans	Cherel et al. (2002b) ; Cherel et al. (2006)
Thin-billed prion (*Pachyptila belcheri*)	TBP	26	0.15	oceanic; sea surface	Antarctic waters, oceanic	High Antarctic	crustaceans	Cherel et al. (2002b) ; Cherel et al. (2006)
Hydrobatidae								
Wilson's storm petrel (*Oceanites oceanicus*)	WSP	17	0.039	neritic; sea surface	unknown	Subtropics	crustaceans	Ridoux (1994)[a]
Black-bellied storm petrel (*Fregetta tropica*)	BBSP	20	0.053	neritic, oceanic; sea surface	unknown	Subtropics	crustaceans and carrion (squid, fish)	Ridoux (1994)[a]
Grey-backed storm petrel (*Garrodia nereis*)	GBSP	18	0.033	neritic; sea surface	unknown	Subtropics	crustaceans	Ridoux (1994)[a]
Pelecanoididae								
Common diving petrel (*Pelecanoides urinatrix*)	CDP	23	0.14	neritic (closed sea); epipelagic	unknown	Subantarctic, low Antarctic	crustaceans	Bocher et al. (2000)
South Georgian diving petrel (*Pelecanoides georgicus*)	SGDP	20	0.12	oceanic; epipelagic	unknown	Subantarctic, low Antarctic	crustaceans	Bocher et al. (2000)
Phalacrocoracidae								
Kerguelen shag (*Phalacrocorax verrucosus*)	KS	65	1.9	neritic (open sea); benthic	resident all year	Neritic waters	benthic fish	Watanabe et al. (2011)
Stercorariidae								
Subantarctic skua (*Catharacta antarctica lönnbergi*)	SS	58	1.9	on land and at sea surface	unknown	Subtropics	small petrels	Mougeot et al. (1998)
Laridae								
Kelp gull (*Larus dominicanus*)	KG	60	1.1	on land and at sea surface	resident all year	Coastal	carrion/seabirds (limpets)	Stahl and Mougin (1986)[a]
Chionididae								
Lesser sheathbill (*Chionis minor*)	LS	40	0.83	on land	resident all year	Terrestrial/coastal	carrion, eggs, invertebrates, algae	Jouventin et al. (1996)
Anatidae								
Kerguelen Pintail (*Anas eatoni eatoni*)	KPi	40	0.48	on land	resident all year	Terrestrial	vegetation	unpublished data

[a] Species average for size (length from the tip of the bill to the end of the tail) and mass (body weight) from Shirihai et al (2002).
[b] Stahl and Mougin (1986) and Ridoux (1994) refer to the related Crozet Islands.

Supplementary Table S2

Adult feather Hg concentrations within the Kerguelen avian community. Abbreviations: Abb., species name abbreviation; Ad:Ch ratio, Adult to chick feather Hg ratio using Hg data from the present work and from Blévin et al. (2013).

Species	Abb.	Year	n	Feather Hg (µg g^{-1} dw)				Ad:Ch ratio
				Mean ± SD	Median	Min–Max	CV (%)	
Spheniscidae								
King penguin (*Aptenodytes patagonicus*)	KP	2007	12	2.22 ± 0.59	2.04	1.45–3.21	26.58	2.0
Gentoo penguin (*Pygoscelis papua*)	GP	2007	12	5.85 ± 3.00	6.15	1.28–9.43	51.33	2.4
Macaroni penguin (*Eudyptes chrysolophus*)	MP	2007	12	2.24 ± 0.29	2.26	1.87–2.75	12.78	6.3
Southern rockhopper penguin (*Eudyptes chrysocome filholi*)	SRP	2007	12	1.96 ± 0.41	1.96	1.22–2.62	20.91	7.3
Diomedeidae								
Wandering albatross (*Diomedea exulans*)	WA	2006	12	16.59 ± 3.78	16.99	9.84–24.13	22.81	3.7
Black-browed albatross (*Thalassarche melanophrys*)	BBA	2005	33	4.07 ± 1.78	3.20	1.71–7.75	43.76	1.6
Light-mantled sooty albatross (*Phoebetria palpebrata*)	LMSA	2005, 2007	16	9.47 ± 3.78	10.12	2.31–14.22	39.92	3.6
Procellariidae								
Northern giant petrel (*Macronectes halli*)	NGP	2008	18	16.26 ± 7.27	14.61	7.99–32.11	44.72	3.1
Grey petrel (*Procellaria cinerea*)	GrP	2005	16	8.71 ± 6.47	6.48	2.22–25.56	74.29	2.8
White-chinned petrel (*Procellaria aequinoctialis*)	WCP	2005	14	5.54 ± 2.24	4.92	2.82–9.38	40.36	3.0
Great-winged petrel (*Pterodroma macroptera*)	GWP	2005	14	15.82 ± 4.44	15.00	9.76–27.13	28.09	9.6
White-headed petrel (*Pterodroma lessonii*)	WHP	2002	10	12.43 ± 2.01	12.56	9.22–17.06	16.21	8.1
Soft-plumaged petrel (*Pterodroma mollis*)	SPP	2010, 2011	19	12.21 ± 4.23	12.44	4.67–25.48	34.63	-
Kerguelen petrel (*Aphrodroma brevirostris*)	KeP	2007, 2008	24	2.27 ± 2.11	1.67	0.62–10.89	93.13	2.9
Blue petrel (*Halobaena caerulea*)	BP	2003	25	1.05 ± 0.72	0.86	0.43–3.32	68.01	1.3
Antarctic prion (*Pachyptila desolata*)	AP	2008	10	1.73 ± 0.50	1.53	1.28–2.61	28.94	8.3
Thin-billed prion (*Pachyptila belcheri*)	TBP	2003	20	0.63 ± 0.55	0.41	0.24–2.49	87.05	2.9
Hydrobatidae								
Wilson's storm petrel (*Oceanites oceanicus*)	WSP	2005	12	0.42 ± 0.13	0.40	0.27–0.68	31.42	-
Black-bellied storm petrel (*Fregetta tropica*)	BBSP	2005, 2010	10	4.22 ± 2.53	3.78	1.46–9.53	59.91	-
Grey-backed storm petrel (*Garrodia nereis*)	GBSP	2006	23	0.51 ± 0.44	0.37	0.22–2.39	87.16	-
Pelecanoididae								

Common diving petrel (*Pelecanoides urinatrix*)	CDP	2003	29	1.06 ± 0.54	0.95	0.35–2.43	51.10	9.4
South-Georgian diving petrel (*Pelecanoides georgicus*)	SGDP	2009, 2010	24	1.07 ± 0.69	0.99	0.10–3.05	64.18	20.7
Phalacrocoracidae								
Kerguelen shag (*Phalacrocorax verrucosus*)	KS	2005	30	5.30 ± 2.33	4.83	1.80–9.71	44.00	2.4
Stercorariidae								
Subantarctic skua (*Catharacta antarctica lönnbergi*)	SS	2009	26	2.65 ± 2.87	1.84	0.39–13.38	108.62	0.5
Laridae								
Kelp gull (*Larus dominicanus*)	KG	2010	5	6.33 ± 5.07	7.63	0.68–12.32	80.00	6.7
Chionididae								
Lesser sheathbill (*Chionis minor*)	LS	2009, 2010	26	1.49 ± 0.92	1.29	0.32–3.56	61.35	-
Anatidae								
Kerguelen pintail (*Anas eatoni eatoni*)	KPi	2010	16	1.69 ± 1.04	1.52	0.36–4.13	61.32	-

[a] References: Marchant and Higgins (1990), Shirihai et al. (2002).

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Paper 4

Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators

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Wandering Albatrosses Document Latitudinal Variations in the Transfer of Persistent Organic Pollutants and Mercury to Southern Ocean Predators

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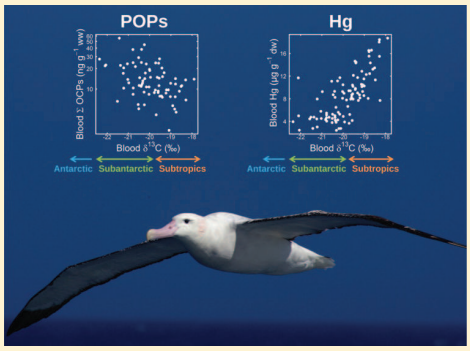
Supporting Information

ABSTRACT: Top marine predators are effective tools to monitor bioaccumulative contaminants in remote oceanic environments. Here, we used the wide-ranging wandering albatross *Diomedea exulans* to investigate potential geographical variations of contaminant transfer to predators in the Southern Ocean. Blood concentrations of 19 persistent organic pollutants and 14 trace elements were measured in a large number of individuals (N = 180) of known age, sex and breeding status from the subantarctic Crozet Islands. Wandering albatrosses were exposed to a wide range of contaminants, with notably high blood mercury concentrations. Contaminant burden was markedly influenced by latitudinal foraging habitats (inferred from blood $\delta^{13}C$ values), with individuals feeding in warmer subtropical waters having lower concentrations of pesticides, but higher concentrations of mercury, than those feeding in colder subantarctic waters. Sexual differences in contaminant burden seemed to be driven by gender specialization in feeding habitats, rather than physiological characteristics, with females foraging further north than males. Other individual traits, such as adult age and reproductive status, had little effect on blood contaminant concentrations. Our study provides further evidence of the critical role of global distillation on organic contaminant exposure to Southern Ocean avian predators. In addition, we document an unexpected high transfer of mercury to predators in subtropical waters, which merits further investigation.



INTRODUCTION

Anthropogenic activities have released intentionally and nonintentionally hundreds of thousands of different chemical compounds into the environment. Among these, persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs), polychlorinated byphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) are of great concern, because they were designed to resist degradation for their agricultural and industrial applications.[1,2] Additionally, human activities have increased emissions to the environment of natural trace elements, such as metals and metalloids, raising their concentrations in a variety of ecosystems worldwide.[3] POPs and both essential and nonessential trace elements can be toxic for humans and wildlife.[4,5] Moreover, some environmental contaminants, such as POPs and mercury (Hg), bioaccumulate in biota and biomagnify up food webs.[6,7] POPs and Hg also undergo long-range atmospheric transport and deposition,[8,9] contributing to the nonpoint source contamination of remote environments. The World Ocean in particular appears to be the last sink for some POPs and trace elements,[8,10,11] but few data are available on their distribution and trophic transfer in remote oceanic areas.[12,13] This is particularly true for the southern Indian Ocean, where POPs[14−18] and trace elements[19−28] have been measured in only some biota, while information on water concentrations lacks dramatically.[12,29,30]

While direct assessment of environmental contaminants in large open water regions is logistically challenging, great insight

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into patterns of marine contamination can be obtained by using top predators as bioindicators.[31] Seabirds, in particular, have revealed important geographical trends of contaminant transfer to predators in a variety of ecosystems.[32,33] Yet, many different factors can drive variation in seabird exposure, hampering their use as reliable bioindicators of marine contamination.[34] Variability in seabird POP and trace element concentrations results not only from extrinsic factors, such as feeding habitat and trophic position,[27,35,36] but also from intrinsic factors, such as detoxification capability and nutritional condition.[2,37] Intraspecific variation in contaminant exposure has received substantial consideration (e.g., ref 38), yet marked between-individual differences often remain largely unexplained.[39,40] Overall, there is a need for more studies that concurrently assess a wide range of causal ecological factors. In particular, life-history traits have been rarely considered,[41,42] due to the paucity of long-term surveys on seabird populations giving access to individuals of known age and breeding history.[28,43,44]

The present study evaluates POP and trace element concentrations in a large number of known individual wandering albatrosses *Diomedea exulans*, breeding at the subantarctic Crozet Islands, southern Indian Ocean. Blood was used as monitoring tissue, since circulating contaminants are known to reflect internal tissues concentrations.[45,46] The wandering albatross is an extremely long-lived (>50 years) cephalopod-eating seabird with one of the highest trophic levels among marine consumers of the Southern Ocean.[24,47] This top predator is thus potentially exposed to large quantities of contaminants through bioaccumulation mechanisms, as shown for Hg.[24,48,49] During the breeding period (a whole year) wandering albatrosses are central-place foragers and undertake large scale movements, foraging up to 3500 km from their nest, thus ranging from subtropical to Antarctic waters.[50,51] This provides an exceptional opportunity to investigate contaminant trophic transfer over a large latitudinal range from one single species of apex predator.

The objectives of this investigation were 3-fold. First, contaminant concentrations of the wandering albatross were described and compared to those found in closely related species worldwide, in order to set results from this study in a global context. Second, by combining information on individual traits from a long-term capture-mark−recapture survey and by using the stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) as trophic tracers, we assessed the relative contribution of intrinsic (sex, age, and breeding status) and extrinsic (feeding ecology) factors on contaminant burden. The final aim of this study was to infer potential latitudinal trends in contaminant transfer to predators in the southern Indian Ocean. Feeding ecology was expected to be more relevant than intrinsic traits in explaining between-individual variation in contaminant concentrations.[27,48] Both POP and Hg burdens were predicted to be higher in individuals feeding in cold subantarctic waters than in those feeding in the subtropics, since polar environmental characteristics (e.g., low temperature, winter darkness) favor the atmospheric deposition and persistence of these contaminants.[1,8,9]

MATERIALS AND METHODS

Study Site and Sampling Procedure. The study was carried out on Possession Island, Crozet Archipelago (46°S, 52°E). The island lies in the Subantarctic Zone that corresponds to the water masses situated between the northern and warmer Subtropical Zone and the southern and colder Antarctic Zone.[52] Adult wandering albatrosses return to their breeding grounds in December and females lay a single egg in late December to early January. Both parents incubate alternatively until hatching in March and most young are fledged in November. During the incubation period (21 December to 4 March 2008), a total of 180 wandering albatrosses were sampled, including breeding and nonbreeding individuals. All birds were of known age (3−49 years), sex and breeding status, since they are part of a long-term capture-mark−recapture program started in 1966.[53] Blood was taken from the tarsal vein with a 1 mL heparinized syringe and a 25-gauge needle. Plasma and red blood cells were separated by centrifugation and stored at −20 °C. POPs and trace elements were measured in plasma and red blood cells, respectively, where they preferentially partition.[49,54,55] Hence, "blood" within the whole text refers either to plasma for POPs or red blood cells for trace elements.

POP and Trace Element Analyses. POPs were measured at the laboratory EPOC-LPTC, Bordeaux, France, from plasma (N = 128, 100 μL aliquots). Targeted compounds included seven indicator PCBs (CB-28, -52, -101, -118, -138, -153, and -180), 11 OCPs (hexachlorobenzene: HCB; lindane: γ-HCH; heptachlor; cis-chlordane; trans-nonachlor; mirex; 2,4′-DDE; 4,4′-DDE; 4,4′-DDD; 2,4′-DDT; 4,4′-DDT) and one PBDE (BDE-47). POPs were quantified using gas chromatography coupled with electron capture detection (GC-ECD). The percentage of total lipids in plasma was also measured on an aliquot of 10 μL by the sulfo-phospho-vanillin (SPV) method for colorimetric determination.[56] POP results are given in both absolute concentrations in ng g^{-1} wet weight (ww) and relative to the plasma lipid weight (lw).

Fourteen trace elements were measured on lyophilized red blood cells at the laboratory LIENSs, La Rochelle, France. Total Hg was quantified with an Altec AMA 254 spectrophotometer (N = 169, aliquots mass: 5−10 mg dry weight, dw), while arsenic (As), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn) were analyzed using a Varian Vista-Pro ICP-OES and silver (Ag), cadmium (Cd), cobalt (Co), lead (Pb), and vanadium(V) using a Series II Thermo Fisher Scientific ICP-MS (N = 165, aliquots mass: 20−200 mg dw). Results are presented in absolute concentrations in μg g^{-1} dw.

Quality Control/Quality Assessment and other details about POP and trace element analyses are given in the Supporting Information (SI) (first paragraph and Table S1). All results are given as means ± SD. Since POP distributions were asymmetric, especially for PCBs, median rather than mean values were used for comparisons with the literature.

Stable Isotope Method. The isotopic niche of albatrosses was used as a proxy of their ecological niche.[57] The isotopic method was validated in the southern Indian Ocean, with δ^{13}C values of seabirds indicating their foraging habitats[50,58] and their δ^{15}N values increasing with trophic level.[59] The isotopic method is based on time-integrated assimilated food, with different tissues recording trophic information at different time scales. In this study, δ^{13}C and δ^{15}N values were measured in red blood cells, which provide trophic information on a few weeks before sampling,[60] thus corresponding here to the incubation period. The effect of blood δ^{13}C and δ^{15}N values on contaminant exposure was investigated using an isotopic data set that was built to study the effect of age, sex and breeding status on foraging strategies of the wandering albatross.[61]

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Table 1. Model Selection for Blood Σ_7PCBs, Σ_{11}OCPs, Hg, and Cd Concentrations in Breeding Wandering Albatrosses from the Crozet Islands[a]

models	k[b]	AIC_c	ΔAIC_c	w_i[c]	exp. var. (%)[d]
$\sum_7$ PCBs-GLM, Gamma Distribution, Inverse Link Function, N = 75 (M: 41, F: 34)					
lipid	2	452	0.00	0.29	7
lipid + age	3	452	0.39	0.24	7
lipid + δ^{13}C	3	453	0.68	0.20	7
lipid + sex	3	453	1.05	0.17	7
age	2	456	3.99	0.04	2
null	1	457	4.62	0.03	
δ^{13}C	2	458	6.09	0.01	0
sex	2	458	6.58	0.01	0
maximal: δ^{13}C + sex + age + lipid + δ^{13}C:sex + δ^{13}C:age	7	459	7.07	0.01	5
$\sum_{11}$ OCPs-GLM, Gamma Distribution, Inverse Link Function, N = 75 (M: 41, F: 34)					
δ^{13}C + sex + lipid	4	503	0.00	0.29	24
δ^{13}C + sex + lipid + δ^{13}C:sex	5	504	1.22	0.16	24
δ^{13}C + sex	3	505	1.46	0.14	21
δ^{13}C + sex + lipid + age	5	505	1.81	0.12	23
δ^{13}C + sex + age	4	506	2.61	0.08	21
δ^{13}C	2	506	3.03	0.06	20
δ^{13}C + lipid	3	506	3.09	0.06	22
Sex	2	507	3.82	0.04	19
δ^{13}C + age	3	507	3.83	0.04	21
maximal: δ^{13}C + sex + age + lipid + δ^{13}C:sex + δ^{13}C:age	7	509	5.74	0.02	22
null	1	521	17.99	0.00	
lipid	2	522	19.20	0.00	0
age	2	523	20.15	0.00	0
Hg-LM, N = 95 (M: 50, F: 45)					
δ^{13}C + sex + δ^{13}C:sex	4	1784	0.00	0.59	53
δ^{13}C + sex + age + δ^{13}C:sex	5	1786	2.27	0.19	53
maximal: δ^{13}C + sex + age + δ^{13}C:sex + δ^{13}C:age	6	1788	4.35	0.07	53
δ^{13}C	2	1789	4.70	0.06	50
Hg-LM, N = 95 (M: 50, F: 45)					
δ^{13}C + sex	3	1789	5.21	0.04	50
δ^{13}C + age	3	1790	6.42	0.02	50
δ^{13}C + sex + age	4	1791	7.03	0.02	50
δ^{13}C + age + δ^{13}C:age	4	1792	7.74	0.01	50
δ^{13}C + sex + age + δ^{13}C:age	5	1793	8.58	0.00	50
sex	2	1820	36.21	0.00	30
sex + age	3	1822	38.39	0.00	29
null	1	1853	69.25	0.00	
age	2	1855	71.22	0.00	0
Cd-GLM, Gamma Distribution, Inverse Link Function, N = 93 (M: 49, F: 44)					
δ^{13}C + sex	3	890	0.00	0.33	7
Sex	2	891	0.94	0.21	5
δ^{13}C + sex + age	4	892	1.99	0.12	6
δ^{13}C + sex + δ^{13}C:sex	4	892	2.17	0.11	6
sex + age	3	893	2.96	0.07	4
δ^{13}C + sex + age + δ^{13}C:age	5	893	3.84	0.05	4
δ^{13}C + sex + age + δ^{13}C:sex	5	894	4.11	0.04	4
null	1	895	4.99	0.03	
maximal: δ^{13}C + sex + age + δ^{13}C:sex + δ^{13}C:age	7	896	6.12	0.02	5
age	2	896	6.82	0.01	0
δ^{13}C	2	896	6.89	0.01	0
δ^{13}C + age	3	898	8.83	0.00	0
δ^{13}C + age + δ^{13}C:age	4	899	10.28	0.00	0

[a]Models are sorted by increasing ΔAIC_c (i.e., decreasing model fit). Abbreviations: AIC_c, Akaike's Information Criteria adjusted for small sample-sizes; w_i, Akaike's weights; Exp. var., explained variation. [b]Number of parameters. [c]Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00. [d]Explained variation calculated from deviance or variance for GLM and LM, respectively, and adjusted depending on k and N.

Results are given in ‰ as means ± SD. Details about stable isotope analyses are given in the SI.

Statistical Analyses. All statistical analyses were performed using R 2.15.1.[62] Only POP and trace element concentrations that were above the limit of quantification (LoQ) in at least 70% of individuals were included in statistical analyses. For these POPs and trace elements, concentrations below the LoQ were substituted using 0.5·LoQ to avoid missing values distorting the statistical outcomes. Data exploration was carried out following Zuur et al.[63] with relationships between variables being tested with Pearson or Spearman correlation tests. In a first descriptive step, a principal component analysis (PCA) was carried out on log-transformed POPs and trace elements in order to highlight covariance. In a second explanatory step, univariate analyses (linear models, LM, or generalized linear models, GLM) were used to test the effect of individual traits and foraging ecology on absolute contaminant concentrations of breeding individuals. As sample sizes differed for POPs and trace elements, separate models were constructed. In order to reduce multiple testing, only the sum of PCBs ($\sum_7$PCBs) and OCPs ($\sum_{11}$OCPs) and the nonessential, potentially harmful Hg and Cd were retained as response variables. Pb and Ag were not considered as response variables, because the former had quantifiable concentrations in less than 70% of individuals, and the latter explained poorly the total variation in the PCA data set (see variable loadings on principal component axis in Table S2, SI). The $\sum_7$PCBs and sumΣ_{11}OCPs were correlated to individual PCBs and OCPs, respectively (Pearson correlation, $0.48 < r < 0.94$ for PCBs and $0.28 < r < 0.97$ for OCPs, all $p < 0.01$, N = 83), with the exception of 2,4′ DDT and heptachlor for OCPs ($r = 0.12$ and 0.05, $p = 0.29$ and 0.62, respectively). Only biologically meaningful models were constructed, with the maximal model being *Contaminant* ~ δ^{13}C + sex + age + δ^{13}C:sex + δ^{13}C:age (with ":" indicating interactions). The percentage of lipids in plasma (hereafter lipid) was also included as a covariate in models explaining $\sum_{11}$OCPs and $\sum_7$PCBs values. Lipid content was not related to feeding habitat or age (data not shown). Blood δ^{15}N was not included in the models, since it was strongly correlated to δ^{13}C (Pearson correlation, $r = 0.85$, $p < 0.0001$, $N = 104$). This results from the slight, latitudinal enrichment in δ^{15}N values from cold to warm waters of the southern Indian Ocean (see also ref 50). Over the large latitudinal gradient exploited by wandering albatrosses, the trophic-level information on δ^{15}N values is thus confounded by a feeding habitat effect. Forward selection using the Akaike's Information Criterion corrected for small sample sizes (AICc)[64] was applied. Since our aim was to make inference on the variables affecting contaminant burdens, the effect of variables was inferred through Akaike's weights, and without using model averaging.[64] Finally, the effect of breeding

status was separately tested on males only, since the sample of nonbreeding individuals was unbalanced (only three nonbreeding females were analyzed for contaminant concentrations). Breeding status categories thus included male immature (3−11 year-old birds with no known breeding attempts), breeding and nonbreeding individuals. GLMs were constructed in the form *Contaminant* ~ Breeding status (+ lipid, for $\sum_{11}$OCPs and $\sum_{7}$PCBs) and hypothesis testing was applied (likelihood ratio test, LRT, between each model and the null model). For all analyses, model specification and validation were based on residuals analysis.[65]

RESULTS

POP and Trace Element Concentrations and Associations. Among the 33 targeted POPs and trace elements, 30 were detected in blood of wandering albatrosses from the Crozet Islands (see Table S3 and S4, SI). The POP pattern was dominated by OCPs (58% of $\sum_{19}$POPs), with the highest median concentrations being reported for 4,4′-DDE and HCB (5.4 and 1.8 ng g^{-1} ww, respectively). Other compounds with quantifiable concentrations in most individuals (>70%) were 4,4′-DDD, mirex and trans-nonachlor. Noticeably, the isomers 2,4′-DDT and 4,4′-DDT had quantifiable concentrations in more than 60% of individuals. The $\sum_{11}$OCPs ranged from 1.3 to 56 ng g^{-1} ww. Indicator PCBs accounted for 40% of the $\sum_{19}$POPs, with congeners CB-138, CB-153, and CB-180 having quantifiable concentrations in most individuals (>70%). The highest median concentration was however reported for CB-118 (2.3 ng g^{-1} ww). The Σ_{7}PCBs ranged from 0.1 to 676 ng g^{-1} ww. Only 18% of individuals had quantifiable concentrations of BDE-47, with values ranging from < LoQ to 1.9 ng g^{-1} ww (BDE-47 accounted for 2% of $\sum_{19}$POPs). Blood POP concentrations presented large between-individual variation, with coefficients of variation (CVs) being particularly high for PCBs (range 119−296%, Table S3, SI).

Among the 14 trace elements, only three were not detected in any individual (the essential Co, Mn, and V), while seven were quantifiable in more than 70% of individuals, including both essential (Cu, Fe, Se, Zn) and nonessential (Ag, Cd, Hg) elements. Fe and Se reported the highest concentrations among essential elements (2326 ± 345 and 77 ± 33 μg g^{-1} dw, respectively). Notably, Hg had quantifiable concentrations in all individuals and showed the highest concentrations among nonessential elements (7.7 ± 3.6 μg g^{-1} dw). Between-individual variation was less pronounced for trace elements than POPs, with the nonessential Ag and Pb having the highest CVs (189% and 95%, respectively, Table S4, SI).

PCA analyses included the eight POPs and seven trace elements that had quantifiable concentrations in more than 70% of individuals (see Table S2, S3, and S4, SI). POPs and Hg contributed markedly to the total variation in the data set. Strong associations were identified within PCBs and within OCPs, but the two POP classes were not associated with each other as shown by the PCA circle of correlations (Figure S1, SI). Hg was potentially negatively associated with OCPs, but no association with other metals was clearly identified.

Explanatory Factors of Between-Individual Variation in Exposure. Univariate analyses were applied to disentangle the influence of sex, age, and feeding habitat (δ^{13}C) on $\sum_{7}$PCBs, $\sum_{11}$OCPs, Hg and Cd burdens in breeding wandering albatrosses (Table 1). For the $\sum_{7}$PCBs, multiple models had a similar support (ΔAICs < 2), but explained only 7% of the total variation (Table 1). Plasma lipid content was clearly the most influential variable, as shown by the sum of Akaike's weights across all models (Table 2). $\sum_{7}$PCBs concentrations increased with increasing lipid content (Figure S2, SI). Multiple models had a similar support for blood $\sum_{11}$OCPs concentrations, explaining approximately 20% of the total variation (Table 1). δ^{13}C, sex, and lipid were the most important predictor variables (Table 2). Exposure was negatively related to δ^{13}C values (Figure 1a). Males and females had Σ_{11}OCPs concentrations of 18.4 ± 10.7 and 11.1 ± 6.2 ng g^{-1} ww, respectively.

Table 2. Sum of Akaike's Weights Across All Models of Each Tested Explanatory Variable for Blood Σ_7PCBs, Σ_{11}OCPs, Hg, and Cd Concentrations in Breeding Wandering Albatrosses From the Crozet Islands

	sum of Akaike's weights across all models			
explanatory variables	$\sum_7$ PCBs	$\sum_{11}$ OCPs	Hg	Cd
age	0.29	0.26	0.31	0.31
lipid	0.91	0.65		
sex	0.19	0.85	0.91	0.95
δ^{13}C	0.22	0.97	1	0.68

One single model best described blood Hg data, the LM δ^{13}C + sex + δ^{13}C:sex, with a percentage of explained variation of 53% (Table 1). The sum of Akaike's weights across all models confirmed the strong effect of δ^{13}C and sex on Hg concentrations (Table 2). Unlike the Σ_{11}OCPs, Hg concen-

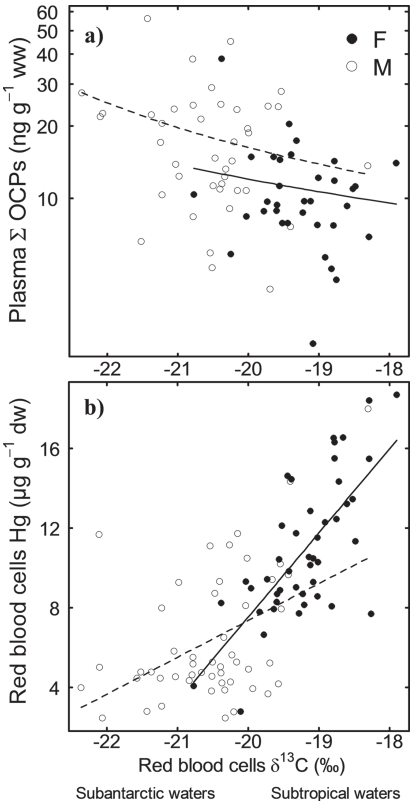

Figure 1. (a) OCP concentrations decrease whereas (b) Hg concentrations increase with decreasing latitude of foraging habitats in blood of breeding wandering albatrosses from the Crozet Islands.

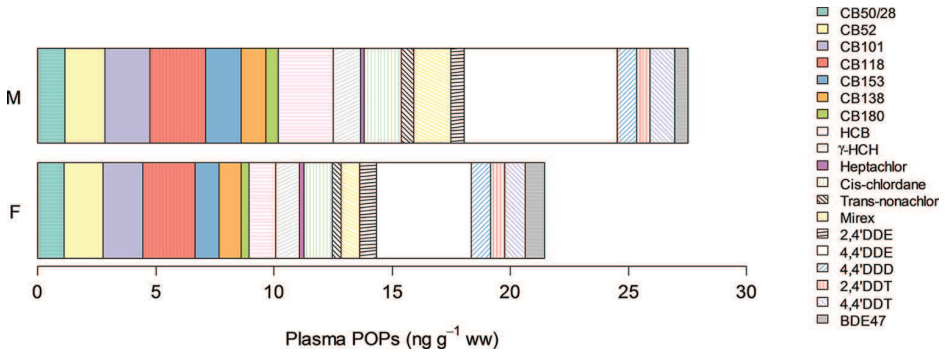

Figure 2. Stacked bar plot of POPs in plasma of male and female wandering albatrosses from the Crozet Islands. Values correspond to median concentrations.

trations were positively related to $\delta^{13}C$ values (Figure 1b) and were lower in males than in females (6.4 ± 3.3 and 10.9 ± 3.5 $\mu g\ g^{-1}$ dw, respectively). Finally, for blood Cd concentrations, multiple models had a similar support, but explained only 5−7% of the total variation (Table 1). Sex was the factor with the highest sum of Akaike's weights across all models (Table 2). Males had lower Cd concentrations than females (56 ± 28 and 72 ± 38 $\mu g\ g^{-1}$ dw, respectively).

Notably, age had no significant effect on contaminant exposure for any of the tested contaminants ($\sum_7$PCBs, $\sum_{11}$OCPs, Hg, and Cd), with the sum of Akaike's weights accounting for age ranging only between 0.26 and 0.31 (Table 2).

Breeding status had a significant effect only on the $\sum_7$PCBs in males (GLM on log-transformed data, Gaussian distribution, identity link function, taking into account the lipid effect, p = 0.01, N = 79), with concentrations in nonbreeding individuals being higher than in breeding ones. Conversely, $\sum_{11}$OCPs, Hg and Cd concentrations were not influenced by breeding status (GLM, gamma distribution, inverse link function, p = 0.38, 0.14, and 0.55, N = 79, 106, and 100, respectively). There was however a tendency for immature birds to show higher blood Hg concentrations than nonbreeding ones.

■ DISCUSSION

The present work is one of the most comprehensive evaluations of POP and trace element burdens in free-living seabirds of known life-history traits, because of the large number of both sampled individuals (N = 180) and targeted contaminants (N = 33). Our results document that the wandering albatross was exposed to a wide range of organic and inorganic contaminants during the breeding period, highlighting the extent of global contamination in the remote Southern Ocean. Recent investigations on physiological and demographic consequences of selected contaminants on the same individuals have revealed that blood POP and Hg concentrations are related to increased oxidative damage[43] and to decreased breeding probability and output.[44]

Pattern of Contamination and Comparison to Other Species and Areas. Previous studies in subantarctic seabirds evaluated legacy-POP concentrations in internal tissues of dead individuals,[14−16,66] and emerging-POPs (perfluorinated compounds) in blood.[17] Therefore, no previous data is available for comparing wandering albatross blood concentrations of legacy-POPs to those of neither subantarctic, nor subtropical seabirds from the Southern Hemisphere. Plasma POP concentrations of the wandering albatross were similar to or lower than those of high-Antarctic seabirds. For example, the south polar skua *Catharacta maccormicki* had three- and 15-fold higher plasma HCB and mirex concentrations, respectively, than the wandering albatross, while median PCB concentrations were comparable.[67] The snow petrel *Pterodroma nivea* showed similar OCP, but higher PCB concentrations than wandering albatrosses.[68] When compared to seabirds from the Northern Hemisphere, wandering albatrosses had overall lower plasma POP concentrations. In particular, two- to 200-fold higher median PCB concentrations have been reported in the plasma of Arctic seabirds (e.g., refs 67 and 69) and North Pacific albatrosses.[13,32,70] Conversely, differences in plasma OCP concentrations between the wandering albatross and Northern Hemisphere seabirds are less pronounced, especially for HCB and mirex.[38,69] Overall, the pattern of organic contamination in plasma of the wandering albatross is remarkable for three main reasons: (1) the smaller abundance of PCBs over OCPs (Figure 2) with respect to Northern Hemisphere species, which is probably related to the distance to industrial sources;[67] (2) the lower concentrations of PBDEs than both PCBs and OCPs, as reported in other albatrosses;[13] and (3) the abundance of HCB, mirex and DDT derivatives, which testifies to their use and emissions in the Southern Hemisphere,[71,72] including recent DDT application for disease vector control.[73]

With regards to nonessential trace elements, blood Hg concentrations were remarkably high in the wandering albatross, as previously shown in feathers (e.g., ref 27, 48, and 49) and internal tissues (e.g., ref 74). Similar blood Hg concentrations have recently been reported in the brown skua *Stercorarius lonnbergi* from the subantarctic Kerguelen Islands.[28] Wandering albatrosses had higher blood Hg concentrations than Antarctic seabirds, such as the south polar skua[28] and the snow petrel.[42] Notably, blood Hg concentrations of wandering albatrosses were comparable to those of the great skua *Catharacta skua*,[39] one of the species with the highest blood Hg concentration in the Northern Hemisphere. On the other hand, blood Cd concentrations were lower than expected, given the importance of squid in the diet of the wandering albatross.[75] Squid has been recognized to be an important vector for Cd transfer to top predators.[19] However, similar low Cd concentrations were found in blood of wandering albatrosses at South Georgia, southern Atlantic Ocean.[35] This result suggests that after assimilation Cd is efficiently transported toward target tissues where it is stored, as proved by high concentrations in liver and kidneys.[74,76] Low blood concentrations were reported also for Pb, which is consistent with results of Anderson et al.[35] and likely the consequence of

low dietary exposure. Indeed, high blood Pb concentrations have been reported in procellariiform species feeding in neritic waters off Patagonia and Brazil, respectively, thus reflecting the contamination of those coastal waters.[77,78] With regards to essential elements, blood concentrations had relatively low between-individual variation (Table S4, SI) and were overall within the same range of values as other subantarctic procellariiform seabirds,[35,77] suggesting that no apparent deficiencies were present in this population.

Effect of Lipids and Breeding Status. Since POPs are strongly lipophilic, their concentrations in living organisms are influenced by lipid dynamics.[2] Here, plasma POP concentrations were influenced by lipid content, in particular for PCBs. This is consistent with previous works showing that lipid status is more determinant for some compounds, such as low-chlorinated PCBs.[41,45,46] Breeding status implies particular physiological conditions that could also affect blood contaminant concentrations.[40,79] Again, only plasma PCBs were significantly affected by breeding status, indicating that physiological traits may be more important than extrinsic factors in driving variation for this class of POPs. On the other hand, immature birds had slightly higher blood Hg concentrations than nonbreeding individuals. It has been hypothesized that immatures moult less frequently than adults,[80] which would mean that they have fewer opportunities to excrete Hg into feathers,[49] but there is no conclusive evidence to support this explanation. Other between-individual and between-compounds differences in detoxification capabilities can also be responsible for the high variability in the data, especially for POPs. The whole body biological half-life of some POPs in birds can be long (100−400 days for the herring gull *Larus argentatus*).[81] There is thus a partial temporal uncoupling between POPs and stable isotope ratios in blood,[6] which could imply carry-over effects of past exposure over wintering grounds.[36] Clearly, a better knowledge on the toxicokinetics of contaminants in blood is needed for the complete understanding of between-individual variation in seabird exposure and contaminant burdens.

Effect of Age. Blood concentrations of POPs, Hg and Cd were not age-related in breeding wandering albatrosses, despite the large age range (7−47 years) and sample size. The absence of age-dependent variation in contaminant concentrations has already been reported in blood[41,49,70,77] and feathers[82,83] of other known-age seabirds, with also some possible decreasing trends being observed.[42] While some studies show contrasting results for age-related trends of trace elements in seabird internal organs, especially Cd,[84,85] overall there is no evidence of a clear, significant increase in POP and Hg concentrations with adult age in any tissue. This pattern could be explained by efficient detoxification mechanisms in seabirds. Indeed, feather excretion is a well-known mechanism for Hg elimination (e.g., ref 37), which is significant also for organic contaminants (e.g., ref 86). Moreover, POPs can be excreted in preen oil[87] and undergo biotransformation in internal tissues.[2] This contrasts with results in marine mammals, which have shown clear age-dependent increases in adult internal tissues contamination, including blood.[88−90] Moreover, the absence of confounding age-related variation in contaminant concentrations enhances the value of adult seabirds' blood as a reliable biomonitoring tool of environmental contamination.[79,82] It must be noted, however, that the present work is a cross-sectional study, which does not necessarily reveal information on the contamination of individuals as they age.[91] However, similar contaminant concentrations have been reported in seabirds sampled repeatedly in different years.[41,70] Furthermore, POPs, Hg, and Cd did not affect mortality in the wandering albatross,[44] which excludes potential bias from differential survival of the most contaminated individuals.

Effect of Feeding Habitat and Sex during the Breeding Period. Key findings of the present study are the correlations between blood $\delta^{13}C$ values and blood OCP and Hg concentrations (Figure 1). The Southern Ocean is marked by a well-defined latitudinal baseline $\delta^{13}C$ gradient that is reflected in the tissue of consumers.[50,58,92] The wide range of blood $\delta^{13}C$ values thus indicates that wandering albatrosses foraged over a vast latitudinal area during the incubation period. Based on blood $\delta^{13}C$ isoscapes, wandering albatrosses exploited large domains of the Subantarctic and Subtropical Zones.[50] The inverse relationship between blood $\delta^{13}C$ values and OCP concentrations (Figure 1a) therefore strongly suggests a latitudinal pattern in OCP transfer to predators, which *increased* from warm subtropical (high $\delta^{13}C$ values) to cold subantarctic waters (low $\delta^{13}C$ values). Conversely, the direct correlation between blood values and Hg concentrations (Figure 1b) likely indicates the opposite pattern in Hg transfer to predators, which *decreased* from warm subtropical (high $\delta^{13}C$ values) to cold subantarctic (low $\delta^{13}C$ values) waters. Importantly, sexual differences in contaminant exposure were explained by gender specialization in foraging habitats.[61,93] Indeed, male (vs female) wandering albatrosses had higher exposure to POPs (vs Hg) likely because they used more subantarctic (vs subtropical) waters (Figure 1). Gender differences in uptake, metabolism, storage and excretion of organic and inorganic contaminants cannot be excluded, particularly taking into account that a fraction of both POPs and Hg is eliminated through the egg for females.[69,85] However, since the wandering albatross is a biennial species laying only one egg at each breeding attempt,[94] egg transfer alone could hardly explain sexual differences in contaminant concentrations (e.g., refs 2 and 95). Indeed, previous studies in low-fecundity seabird species have shown that sexual differences in contaminant concentrations are not always significant and are in different directions depending on species.[2,77,95] The observed higher blood concentration of Cd in females than in males was partially related to feeding habitats (Table 2) and is more difficult to explain. Nevertheless, different proportions of particular prey species (e.g., squids) between males and females could account for this pattern.

Latitudinal Variation in POP and Hg Transfer to Predators. The observed latitudinal pattern in POP transfer to the albatrosses, increasing from warm subtropical to cold subantarctic waters, is consistent with the cold condensation and fractionation theory. The latter predicts that increasing quantities of POPs are deposited in polar environments by repeated air-surface exchange and atmospheric transport, with the mixture shifting to more volatile compounds.[9] Our results thus suggest that OCPs enter readily trophic webs after atmospheric deposition, likely through adsorption on organic matter particles and uptake by phytoplankton.[10] Global distillation seems thus to significantly affect predators' exposure to OCPs in the Southern Ocean. This was not verified for PCBs, most likely due to the extremely high between-individual variation in blood (see second section of the discussion). Increases in both OCP and PCB exposure at high latitudes have already been observed in different species and populations of seabirds from Antarctica[71] and the Norwegian Arctic,[38,96]

respectively, but we are not aware of previous evidence of such a latitudinal variation within a single seabird population.

Unlike POPs and contrary to our prediction, Hg transfer to the albatrosses decreased from warm subtropical to cold subantarctic waters. Previously, high concentrations of atmospheric Hg have been observed close to the Antarctic continent when compared to lower latitudes.[97] Moreover, the only biogeochemical investigation on Hg speciation and distribution in Southern Ocean waters has shown high concentrations of Me-Hg in Antarctic rather than subantarctic and subtropical waters.[30] Methyl-Hg is the bioavailable and most toxic form of Hg that is readily assimilated by low-trophic level organisms and then biomagnifies up the food web.[7] The heavy Hg burden of birds feeding in warm subtropical waters is therefore puzzling. However, a significant higher exposure to Hg in chicks fed by parents foraging in subtropical rather than subantarctic waters was previously underlined in different oceanic seabird species from the Kerguelen Islands.[24] A similar trend appears also from between-species comparisons. Indeed, Antarctic top predators present lower Hg concentrations than subantarctic ones (see first section of the discussion). Antarctic food webs are simpler that those found at subtropical latitudes.[50,98] Since food web structure influences Hg transfer,[99,100] the complexity of subtropical food webs could explain the higher Hg exposure in northern than southern foraging predators of the Southern Ocean. In addition, Hg dynamics within food webs is affected by many other factors, such as primary productivity, temperature and solar radiation.[8] Clearly, our results call for in-depth investigations of Hg speciation and food web dynamics in waters of the Southern Ocean.

Despite the presence of several confounding factors, our data documents a clear latitudinal trend in both POP and Hg transfer to predators in the southern Indian Ocean. Since breeding wandering albatrosses feed *all along* their foraging trips, their blood isotopic signature integrates prey taken in different water masses, thus diluting $\delta^{13}C$ values.[61] Such a "'dilution effect"' reduces differences among individuals, further emphasizing the strength of the habitat-related contaminant exposure depicted here.

■ ASSOCIATED CONTENT

Supporting Information

Full details on the analytical techniques and Quality Control/Quality Assessment used for persistent organic pollutant (POP), trace element and stable isotope analyses. Results on the effect of heparin on blood mercury (Hg) concentrations. Tables detailing the limits of detection (LoD) and quantification (LoQ), descriptive statistics and principal component (PC) loadings of all the contaminants measured in blood of wandering albatrosses. Figures of the circle of correlations of the PC analyses, and of the relationship between blood Σ_7PCBs concentrations and plasma lipid content in breeding individuals. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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Supporting Information

Title: Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators

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1. Persistent organic pollutant (POP) and trace element analyses

POPs were measured in albatross plasma at the laboratory EPOC-LPTC, Bordeaux, France, (N = 128, 100 µl aliquots). Targeted compounds included seven polychlorinated biphenyls (PCBs: CB-28/50, -52, -101, -118, -138, -153 and -180), 11 organochlorine pesticides (OCPs: HCB, γ-HCH, heptachlor, 2,4' DDE, cis-chlordane, 4,4' DDE, trans-nonachlor, 4,4' DDD, 2,4' DDT, 4,4' DDT, mirex) and one polybrominated diphenyl ether (PBDE: BDE 47). Internal standards (CB-30, -103, -155, -198 for PCBs, p,p'-DDT-d8 for OCPs and F-BDE-47 for BDE 47, 1 ng each) were added to each sample. Standards were provided by either Dr Ehrenstorfer GmbH or Cambridge Isotope Laboratory (*via* Cluzeau Info Labo, Sainte-Foy-La-Grande, France). POPs were extracted with 1 mL of pentane/dichloromethane (90/10; v/v); after centrifugation (2000 rpm, 2 min at 4°C), the organic layer was collected and the operation was repeated. Both extracts were combined and purified on an acid silica gel column (40% H_2SO_4). After extract loading, analytes were eluted with 3 x 5 mL of pentane/dichloromethane (90/10; v/v). The so-obtained extract was then concentrated using a RapidVap vacuum evaporation system from Labconco (Kansas City, MO, USA) to a volume of 1 mL; it was then further concentrated under a gentle stream of nitrogen (40°C) after addition of 100 µL of isooctane as solvent keeper. A syringe standard (octachloronaphtalene, 1 ng) was finally added to quantify internal standards and to assess their recovery rate for each sample (68-108%). Final extracts were analysed by gas chromatography coupled with electron capture detection (GC-ECD) as described in Tapie et al. (2011). CB-28 and -50 co-eluted in all samples. Quality control consisted in the analysis of procedural blanks (clean and empty glass tubes treated like a sample, one run for 8 samples). Chicken plasma samples (Sigma-Aldrich, St Quentin Fallavier, France) spiked at 3 ng g^{-1} were analysed in parallel to the samples; the recovery rates of PCBs and OCPs were in the range 77-103% with coefficients of variation lower than 17% (N = 5), except for CB 52 (22%) and mirex (29%). POP concentrations were blank corrected and the detection limit (LoD) was set at two times the mean blank value; for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of 3. Overall, LoDs ranged from 0.02 to 0.8 ng g^{-1} wet weight, ww (Table S1).

Trace elements were measured on lyophilized red blood cells at the laboratory LIENSs, La Rochelle, France. Total mercury (Hg) was quantified with an Altec AMA 254 spectrophotometer (N = 169, aliquots mass: 5-10 mg dry weight, dw). All analyses were repeated in duplicate-triplicate until having a relative standard deviation < 5% for each

individual. Accuracy was checked using a certified reference material (CRM, Tort-2 Lobster Hepatopancreas, NRC, Canada; certified Hg concentration: 0.27 ± 0.06 µg g^{-1} dw). Our measured values were 0.24 ± 0.02 µg g^{-1} dw, N = 31. Mass of the CRM was adjusted to represent the same amount of Hg introduced in the AMA compared to the one in blood samples. Blanks were analysed at the beginning of each set of samples and the LoD was 0.005 µg g^{-1} dw. Arsenic (As), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn) were analysed using a Varian Vista-Pro ICP-OES and silver (Ag), cadmium (Cd), cobalt (Co), lead (Pb) and vanadium (V) using a Series II Thermo Fisher Scientific ICP-MS (N = 165, aliquots mass: 20-200 mg dw). Lyophilised red blood cells were mineralized before analysis. Measurement quality was assessed by CRMs (Tort-2 Lobster Hepatopancreas and Dolt-4 Dogfish Liver, both NRC, Canada), which were treated and analysed in the same way as the samples. Results were in line with the certified values, and the standard deviations were low, proving good repeatability of the method. Elements recovery of standard reference materials ranged from 84% to 119%. For each set of analyses, blanks were included in each analytical batch. The LoD (µg g^{-1} dw) were 0.015 (Cd), 0.017 (Ag), 0.02 (Cr, Co, Pb), 0.03 (Ni), 0.08 (Mn), 0.1 (Cu, Se), 0.2 (As), 0.3 (V) and 3.3 (Fe and Zn).

2. Effect of heparin on red blood cell mercury concentrations

The effect of heparin on red blood cell Hg concentrations was tested on a domestic pigeon *Columba livia domestica* by sampling blood using heparinized and non-heparinized syringes. Heparinized and non-heparinized samples had identical red blood cells Hg concentrations (0.006 ± 0.001 and 0.006 ± 0.002 µg g^{-1} dw, respectively, n = 4 in duplicate, Wilcoxon test, W = 9, p = 0.89).

3. Stable isotope analyses

The relative abundance of stable isotopes was determined in red blood cells with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112) (N = 158, aliquots mass: ~0.3 mg) at the laboratory LIENSs, La Rochelle, France. Results are in the usual δ notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors < 0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. Results are given in ‰ as means ± SD.

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Table S1. Limit of detection (LoD) and of quantification (LoQ) in ng g^{-1} ww for the targeted POPs.

	Session[1]	CB-28/50	CB-52	CB-101	CB-118	CB-138	CB-153	CB-180
LoD	1	0.1	0.1	0.1	0.2	0.1	0.1	0.02
LoQ		0.2	0.4	0.4	0.7	0.2	0.3	0.08
LoD	2	0.1	0.8	0.6	0.5	0.2	0.3	0.01
LoQ		0.4	2.5	1.9	1.7	0.8	0.9	0.03

	Session[1]	HCB	γ-HCH	Heptachlor	2,4-DDE	4,4-DDE	Cis-chlordane	Trans-nonachlor	4,4'-DDD	2,4'-DDT	4,4'-DDT	Mirex	BDE-47
LoD	1	0.03	0.13	0.03	0.09	0.05	0.07	0.04	0.05	0.04	0.04	0.05	0.05
LoQ		0.09	0.44	0.10	0.31	0.16	0.23	0.15	0.16	0.13	0.13	0.16	0.16
LoD	2	0.17	0.04	0.05	0.15	0.15	0.15	0.15	0.15	0.01	0.05	0.05	0.03
LoQ		0.57	0.13	0.17	0.49	0.49	0.49	0.49	0.49	0.03	0.16	0.17	0.09

[1] POP concentrations were analysed in two sessions, with LoD and LoQ being calculated from the mean blank of the relative session.

Table S2. Blood POP and trace element loadings on the three axes selected from the principal component analysis of wandering albatrosses from the Crozet Islands.

Axis	CB-153	CB-138	CB-180	HCB	Trans-nonachlor	4,4' DDE	4,4' DDD	Mirex	Hg	Fe	Cu	Zn	Se	Ag	Cd
PC1	-0,09	-0,12	-0,04	0,41	0,43	0,45	0,39	0,47	-0,14	0,04	-0,01	-0,01	0,06	0,05	-0,12
PC2	-0,52	-0,50	-0,51	-0,21	0,11	-0,09	-0,03	-0,04	0,24	0,10	0,16	0,14	0,15	0,09	0,08
PC3	0,17	0,19	0,16	0,09	-0,03	0,10	0,02	-0,01	0,12	0,49	0,46	0,39	0,30	0,07	0,42

Table S3. Plasma lipid content and POP concentrations (ng g^{-1}) relative to the wet and lipid weight (ww and lw, respectively) of the wandering albatross from the Crozet Islands (samples above the LoQ).

	N	> LoQ (%)	Mean ± SD		Median		Min – Max		CV (%)
Lipid (%)	128/128	100	0.62 ± 0.13		0.60		0.37 – 1.03		21
			ww	lw	ww	lw	ww	lw	ww
CB 50/28	64/128	50	1.8 ± 2.7	282 ± 421	1.2	173	< LoQ – 19.7	< LoQ – 2983	148
CB 52	86/128	67	7.7 ± 22.7	1191 ± 3544	1.7	271	< LoQ – 192.2	< LoQ – 29120	296
CB 101	70/128	55	12.6 ± 25.9	1926 ± 3979	1.9	289	< LoQ – 174.7	< LoQ – 26470	206
CB 118	82/128	64	13.0 ± 26.5	2011 ± 4101	2.3	363	< LoQ – 176.0	< LoQ – 26660	203
CB 153	112/128	88	4.6 ± 8.5	715 ± 1319	1.4	262	< LoQ – 49.3	< LoQ – 7465	187
CB 138	100/128	78	5.6 ± 11.5	858 ± 1777	1.0	174	< LoQ – 58.9	< LoQ – 8945	207
CB 180	128/128	100	0.8 ± 0.9	127 ± 146	0.5	75	0.10 – 5.7	15 – 867	119
Σ_7 PCBs			**30.4 ± 79.6**	**4705 ± 12282**	**5.7**	**1008**	**0.1 – 676.4**	**15 – 102500**	**261**
HCB	125/128	98	2.2 ± 1.5	357 ± 257	1.8	283	< LoQ – 10.0	< LoQ – 1458	72
γ-HCH	59/128	46	1.7 ± 0.6	206 ± 105	1.1	197	< LoQ – 3.6	< LoQ – 630	50
Heptachlor	37/128	29	0.4 ± 0.8	71 ± 132	0.2	33	< LoQ – 4.7	< LoQ – 712	194
Cis-chlordane	77/128	60	1.5 ± 1.0	250 ± 166	1.4	214	< LoQ – 5.2	< LoQ – 806	66
Trans-nonachlor	99/128	77	0.5 ± 0.3	83 ± 45	0.5	74	< LoQ – 1.6	< LoQ – 269	52
Mirex	122/128	95	1.4 ± 1.1	234 ± 180	1.1	173	< LoQ – 6.2	< LoQ – 946	77
2-4' DDT	84/128	66	1.1 ± 1.4	176 ± 221	0.6	89	< LoQ – 6.8	< LoQ – 1257	123
4-4' DDT	78/128	61	1.4 ± 1.6	228 ± 250	1.0	154	< LoQ – 9.3	< LoQ – 1615	108
2-4' DDE	55/128	43	0.8 ± 0.5	129 ± 90	0.6	100	< LoQ – 2.8	< LoQ – 528	65
4-4' DDE	125/128	98	6.8 ± 5.0	1138 ± 838	5.4	946	< LoQ – 28.9	< LoQ – 4846	73
4-4' DDD	102/128	80	0.9 ± 0.5	154 ± 82	0.8	135	< LoQ – 2.6	< LoQ – 560	50
Σ_{11} OCPs			**14.7 ± 9.0**	**2445 ± 1505**	**12.2**	**2089**	**1.3 – 56.0**	**263 – 7583**	**61**
BDE 47	23/128	18	0.6 ± 0.4	97 ± 64	0.6	94.6	< LoQ – 1.9	< LoQ – 322	66
Σ_{19} POPs			**45.3 ± 81.1**	**7167 ± 12475**	**20.1**	**3253**	**2.3 – 696.2**	**448 – 105500**	**179**

Table S4. Trace element concentrations (µg g^{-1} dw) and stable isotope ratios (‰) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in red blood cells of the wandering albatross from the Crozet Islands.

	N	> LoQ (%)	Median	Mean ± SD	Min – Max	CV (%)
Non-essential trace elements						
Ag	165/165	100	0.3	0.9 ± 1.7	0.1 – 9.4	189
Cd	143/165	87	0.06	0.07 ± 0.03	< LoQ – 0.22	43
Hg	169/169	100	7.5	7.7 ± 3.6	2.0 – 18.7	47
Pb	60/165	36	0.04	0.06 ± 0.06	< LoQ – 0.43	95
Essential trace elements						
As	80/165	48	0.6	0.7 ± 0.4	< LoQ – 2.3	53
Co	All < LoQ	0	-	-	-	-
Cr	64/165	39	0.2	0.2 ± 0.2	< LoQ – 0.9	90
Cu	165/165	100	1.1	1.2 ± 0.3	0.5 – 4.1	27
Fe	165/165	100	2347	2326 ± 345	879 – 5100	15
Mn	All < LoQ	0	-	-	-	-
Ni	21/165	13	0.1	0.1 ± 0.1	< LoQ – 0.5	79
Se	165/165	100	73.4	77.1 ± 33.0	13.6 – 216	43
Zn	165/165	100	21.9	22.2 ± 3.9	8.5 – 41.4	18
V	All < LoQ	0	-	-	-	-
Stable isotope ratios						
$\delta^{13}C$	158/158	100	-19.8	-19.9 ± 0.9	-22.4 – -17.9	-
$\delta^{15}N$	158/158	100	13.8	13.8 ± 0.7	11.8 – 15.4	-

Abbreviations: SD, standard deviation; CV, coefficient of variation; Ag, silver; Cd, cadmium; Hg, mercury; Pb, lead; As; arsenic; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Mn, manganese; Ni, nickel; Se, selenium; Zn, zinc; V, vanadium.

Fig. S1. Circle of correlations with the two first axes (PC1 and PC2) of a principal component analysis of log-transformed, standardized POPs and trace elements in blood of the wandering albatross from the Crozet Islands.

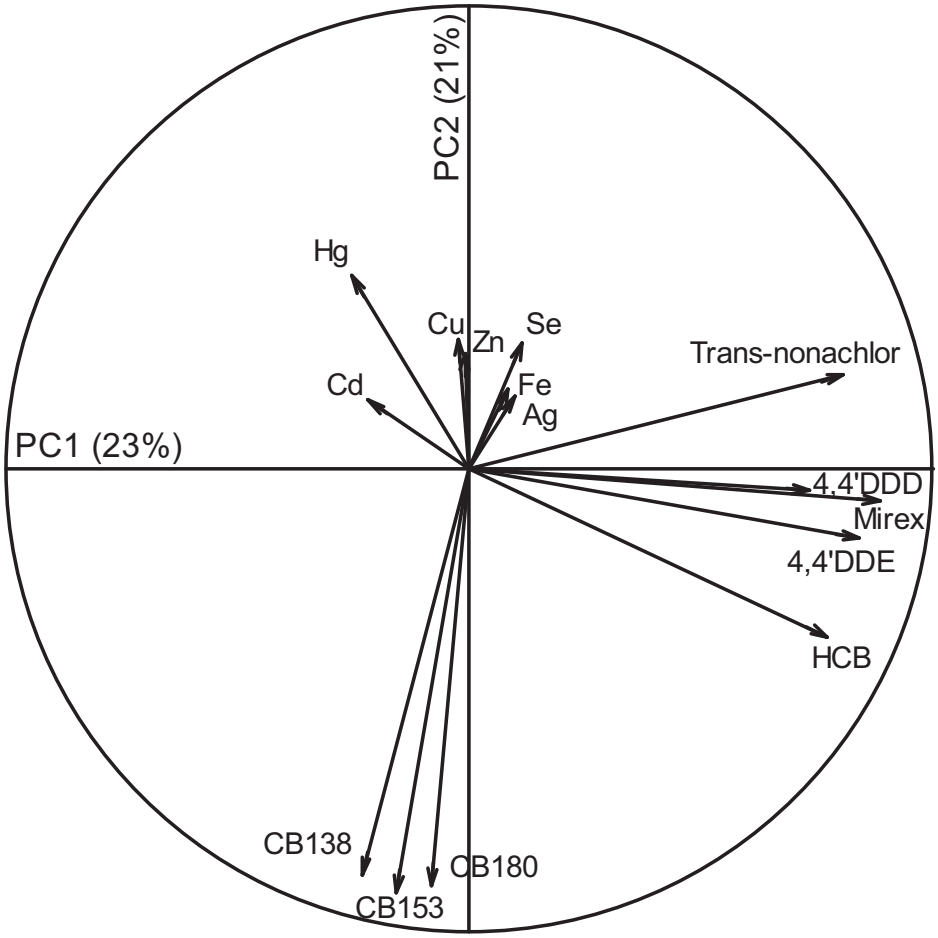
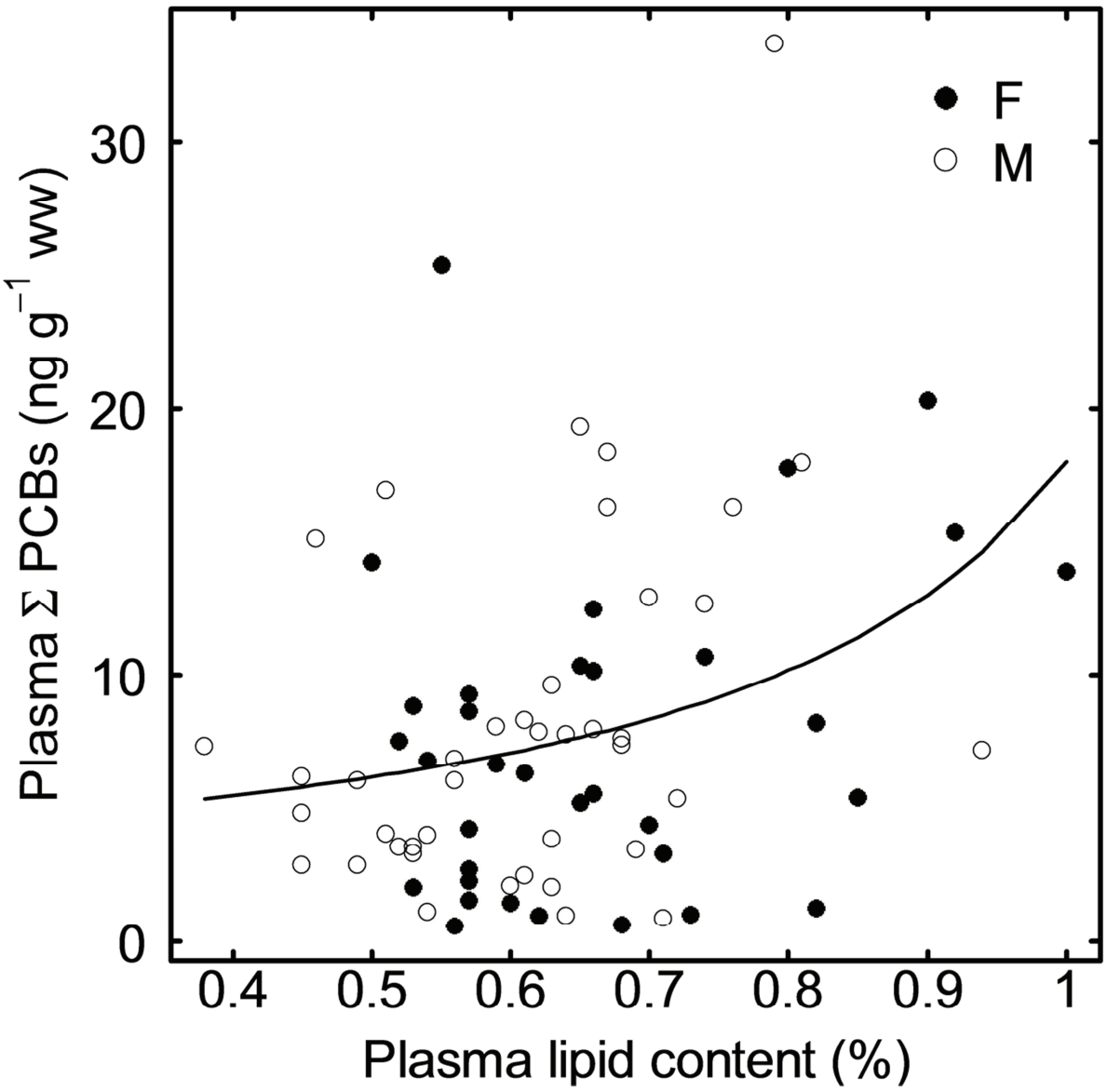


Fig. S2. PCB concentrations increase with lipid content in blood of breeding wandering albatrosses from the Crozet Islands.



Paper 5

Wide range of mercury contamination in chicks of Southern Ocean seabirds

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Wide Range of Mercury Contamination in Chicks of Southern Ocean Seabirds

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Abstract

Using top predators as sentinels of the marine environment, Hg contamination was investigated within the large subantarctic seabird community of Kerguelen Islands, a remote area from the poorly known Southern Indian Ocean. Chicks of 21 sympatric seabirds presented a wide range of Hg concentrations, with the highest contaminated species containing ~102 times more feather Hg than the less contaminated species. Hence, Kerguelen seabirds encompass the whole range of chick feather Hg values that were previously collected worldwide in poorly industrialized localities. Using stable isotopes, the effects of foraging habitats (reflected by $\delta^{13}C$) and trophic positions (reflected by $\delta^{15}N$) on Hg concentrations were investigated. Species-related Hg variations were highly and positively linked to feather $\delta^{15}N$ values, thus highlighting the occurrence of efficient Hg biomagnification processes within subantarctic marine trophic webs. By contrast, Hg contamination overall correlated poorly with feeding habitats, because of the pooling of species foraging within different isotopic gradients corresponding to distinct seabird habitats (benthic, pelagic, neritic and oceanic). However, when focusing on oceanic seabirds, Hg concentration was related to feather $\delta^{13}C$ values, with species feeding in colder waters (lower $\delta^{13}C$ values) south of Kerguelen Islands being less prone to be contaminated than species feeding in northern warmer waters (higher $\delta^{13}C$ values). Within the context of continuous increase in global Hg emissions, Kerguelen Islands that are located far away from anthropogenic sources can be considered as an ideal study site to monitor the temporal trend of global Hg contamination. The present work helps selecting some seabird species as sentinels of environmental pollution according to their high Hg concentrations and their contrasted foraging ecology.

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Introduction

Mercury (Hg) is a highly toxic non-essential metal that negatively influences humans and wildlife [1,2]. In birds, mercury's adverse effects are far-ranging, with impacts on reproduction being usually the end-point of more direct effects on behaviour, neurology, endocrinology and development [2]. Overall, Hg derives from both natural and anthropogenic sources, but human activities have increased the global amount of Hg cycling around the world by a factor of three to five [3]. Owing to its high volatility and long atmospheric residence time, Hg reaches remote areas through long-range atmospheric transport, thus contaminating oceanic islands and polar and sub-polar regions [4,5]. Once deposited in aquatic ecosystems, inorganic Hg is subject to biotic reaction (methylation) carried out by microorganisms [6]. Thereafter, methylmercury (Me-Hg), the persistent and highly toxic form of Hg, is assimilated by living organisms *via* food intake, bioaccumulates in individuals and biomagnifies within food webs from lower to higher trophic levels [7]. Hence, top predators have been used to monitor Hg contamination in various aquatic ecosystems [8], with still limited existing information for significant parts of the world ocean, including the southern Indian Ocean [9].

Seabirds are useful bio-indicators of environmental pollution, including Hg contamination because they are long-lived animals that prey at the top of the marine food webs [8,10]. Feathers are the most attractive avian tissue to sample because, in many cases, they contain most of the Hg body burden and are considered as the main route for Hg elimination [8]. Hg accumulated and stored within soft tissues between moults is transferred and sequestered in the growing feathers and cannot thus be re-incorporated into living tissues. Feather sampling has also the benefit to be non-destructive.

Here, we investigated feather Hg concentrations within the large community of seabirds that breed in Kerguelen Islands, a remote subantarctic archipelago from the Southern Ocean [11]. The seabird assemblage is mainly composed of penguins and Procellariiformes, including the very long-lived and slow-moulting albatrosses that are amongst the most Hg contaminated vertebrate species [12]. Kerguelen seabirds feed on a large diversity of prey (crustaceans, cephalopods and fish) and use contrasted foraging strategies that include benthic and pelagic feeding as well as foraging in neritic and oceanic waters, with the oceanic birds ranging from the warmer subtropical to the colder Antarctic waters (Table 1). The distribution of Hg species in the main oceanic water masses is still poorly

documented, with a complete lack of information from Kerguelen waters. However, a recent investigation on Hg speciation within the Southern Ocean revealed a poleward increase in the concentrations of total Hg and Me-Hg in surface waters to some of the highest Me-Hg concentrations so far observed in the open ocean [13]. This Me-Hg increase most likely results from a strong net Hg methylation in the hypoxic zone of the water column in Antarctic waters [13].

In a first descriptive step, Hg was determined in 21 seabird species to assess contamination levels of marine ecosystems within the poorly known southern Indian Ocean. In a second explanatory step, the effect of differences of seabird foraging ecology on feather Hg concentration was tested, because ingestion of food is the main route of Hg exposure in birds [10]. The respective roles of habitats and diets were tested by using the isotopic niche as a proxy of the trophic niche of the species, with the ratios of stable isotopes of

Table 1. Species, foraging habitats and diets during the chick-rearing period, and durations of the chick rearing period of seabirds at Kerguelen Islands.

Species		Chick rearing	Main foraging habitats		Main prey classes	References
		period (days)	Horizontal	Vertical		
Spheniscidae						
King penguin (*Aptenodytes patagonicus*)	KP	315	oceanic	mesopelagic	mesopelagic fish	[58]
Gentoo penguin (*Pygosce lis papua*)	GP	72	neritic (open sea)	benthic	benthic fish (crustaceans)	[59]
Macaroni penguin (*Eudyptes chrysolophus*)	MP	65	neritic (open sea)/ oceanic	epipelagic	crustaceans and fish	Unpublished data
Southern rockhopper penguin (*Eudyptes chrysocome*)	SRP	71	neritic (closed sea)	epipelagic	crustaceans	[60]
Diomedeidae						
Wandering albatross (*Diomedea exulans*)	WA	275	oceanic	sea surface	benthopelagic fish and cephalopods	Unpublished data
Black-browed albatross (*Thalassarche melanophrys*)	BBA	125	neritic (open sea)	sea surface	benthopelagic fish (cephalopods)	[19]
Light-mantled sooty albatross (*Phoebetria palpebrata*)	LMSA	154	oceanic	sea surface	cephalopods (crustaceans, carrion)	[61][a]
Procellariidae						
Northern giant petrel (*Macronectes halli*)	NGP	113	on land and at sea	on land, sea surface	carrion/seabirds	[61][a]
Grey petrel (*Procellaria cinerea*)	GrP	128	oceanic	sea surface	fish (cephalopods)	Unpublished data
White-chinned petrel (*Procellaria aequinoctialis*)	WCP	96	oceanic	sea surface	fish (cephalopods, crustaceans)	[62]
Great-winged petrel (*Pterodroma macroptera*)	GWP	126	oceanic	sea surface	cephalopods (crustaceans)	[61][a]
White-headed petrel (*Pterodroma lessonii*)	WHP	101	oceanic	sea surface	fish (cephalopods)	Unpublished data
Kerguelen petrel (*Aphrodroma brevirostris*)	KeP	60	oceanic	sea surface	crustaceans	[61][a]
Blue petrel (*Halobaena caerulea*)	BP	55	oceanic	sea surface	crustaceans (mesopelagic fish)	[49]
Antarctic prion (*Pachyptila desolata*)	AP	50	oceanic	sea surface	crustaceans	[63]
Thin-billed prion (*Pachyptila belcheri*)	TBP	50	oceanic	sea surface	crustaceans	[63]
Pelecanoididae						
Common diving petrel (*Pelecanoides urinatrix*)	CDP	54	neritic (closed sea)	epipelagic	crustaceans	[64]
South-Georgian diving petrel (*Pelecanoides georgicus*)	SGDP	55	oceanic	epipelagic	crustaceans	[64]
Phalacrocoracidae						
Kerguelen shag (*Phalacrocorax verrucosus*)	KS	56	neritic (open sea)	benthic	benthic fish	[65]
Stercorariidae						
Subantarctic skua (*Catharacta antarctica lönnbergi*)	SS	45	on land and at sea	on land, sea surface	small petrels	[47]
Laridae						
Kelp gull (*Larus dominicanus*)	KG	49	on land and at sea	on land, sea surface	carrion/seabirds (limpets)	[66][a]

[a]Stahl and Mougin (1986) and Ridoux (1994) refer to the related Crozet Islands.

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carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) reflecting their foraging habitats and trophic positions, respectively [14]. The isotopic method was already validated in the area, with seabird $\delta^{13}C$ values indicating their latitudinal foraging grounds and depicting offshore versus inshore consumers [15,16] and their $\delta^{15}N$ values increasing with trophic level [17]. Taking into account the species' foraging ecology, we make the following predictions. Firstly, feeding habitat ($\delta^{13}C$) should shape seabird Hg contamination, because Hg is not homogeneously distributed in marine ecosystems. For example, (i) benthic foragers should have relatively high feather Hg concentrations in relation to the substantial production of Me-Hg in coastal marine sediments [6], and (ii) oceanic foragers should be more contaminated in cold than in warm waters, in relation to the latitudinal gradient in the bioavailability of Me-Hg in the Southern Ocean [13]. Secondly, Hg should magnify within subantarctic food webs, which means that seabirds with the highest trophic positions ($\delta^{15}N$) should show the highest feather Hg concentrations. Finally, the longer the chick rearing period, the higher the chick feather Hg concentrations should be, because much of the dietary Hg accumulated in soft tissues is mobilized and excreted into growing feathers [18].

Most previous investigations on pollutants in birds were conducted on adults that present some disadvantages related to their foraging areas, moult schedule and migration patterns. Instead, we focused on chicks as indicators and sentinels of contamination [10]. Firstly, young birds that have not yet fledged have obtained all of their food from their parents who forage in the vicinity of the breeding colonies at that time. The chick Hg concentrations and stable isotope signatures thus reflect those of the adult foraging ecology during the chick-rearing period, a period during which feeding information (chick diet and adult foraging grounds) were previously collected using complementary methods (i.e. dietary analysis and bio-logging [19]). Secondly, the integration period is almost identical for Hg and stable isotopes in body feathers of chicks, because (i) Hg accumulated during the chick-rearing period is excreted during the pre-fledging moult, and (ii) feather isotope ratios reflect diet at the time of feather synthesis [20]. Thirdly, since all chick feathers were grown almost simultaneously, both Hg and stable isotopes are fairly homogeneous in the plumage of pre-fledglings [8,21], while sequential moult in adults induces larger Hg concentrations in the first than in the late growing feathers [22].

Materials and Methods

Ethics Statement

Animals were cared for in accordance with the guidelines of the ethics committee of the Institut Polaire Français Paul Emile Victor that approved fieldwork in the present study (Program no. 109, H. Weimerskirch).

Study Area, Species and Sample Collection

Fieldwork was carried out from 2003 to 2012 on Kerguelen archipelago (49°21′S, 70°18′E), which is located in the southern part of the Subantarctic Zone, in the immediate vicinity of the Polar Front [23]. The Southern Ocean was defined as the ocean south the Subtropical Front and the Subantarctic and Antarctic Zones, as the zones between the Subtropical and Polar Fronts, and between the Polar Front and Antarctica, respectively (Fig. 1). Chicks from 21 seabird species belonging to 4 orders and 7 families were sampled, totalizing 280 individuals (n = 7–22 per species). Sampling was conducted at different locations of the archipelago, depending on the species' breeding sites. Most neritic seabirds were studied in colonies close to the open sea, but two species (the southern rockhopper penguin and common diving petrel) were sampled at Mayes Island that is located within a large bay (closed sea) where the parent birds feed. Body feathers were collected from the lower back of large chicks at fledging, i.e. at the end of the breeding season. Once collected, feathers were stored dry in sealed plastic bags and analysed at the University of La Rochelle, France.

Sample Analyses

Prior to chemical analysis, all body feathers were cleaned of surface lipids and contaminants using a 2:1 chloroform: methanol solution for 2 min followed by two successive methanol rinses. They were then cut with scissors into small fragments. A first subsample of homogenized feathers was oven dried for 48 hr at 50°C and then analyzed in an advanced Hg analyzer spectrophotometer (Altec AMA 254). Hg determination involved evaporation of Hg by progressive heating until 800°C under oxygen atmosphere for 2′30 min and subsequent amalgamation on a gold trap. The net was heated to liberate the collected Hg that was measured by UV atomic absorption spectrophotometry. Samples were analysed for total Hg, which approximates the amount of Me-Hg since essentially all Hg in feathers is under organic form [24,25,26]. All analyses were repeated 2–3 times until having a relative standard deviation <10%. Accuracy was checked using certified reference material (Tort-2 Lobster Hepatopancreas, NRC, Canada; mean 0.27 ± 0.06 $\mu g \cdot g^{-1}$ dry mass). Our measured values were 0.24 ± 0.01 $\mu g \cdot g^{-1}$ dry mass, $n = 56$. Blanks were analysed at the beginning of each set of samples and the detection limit of the method was 0.005 $\mu g \cdot g^{-1}$dry mass.

A second sub-sample of homogenized feathers was weighed (~0.3 mg) with a microbalance and packed into a tin container. Relative abundance of C and N isotopes were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific Flash EA 1112). Results are presented in the δ notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors <0.10‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

Statistical Analyses

Statistical tests were performed using R 2.7.1 [27]. All samples submitted to statistical tests were first checked for normality and homogeneity of variances by means of Shapiro-Wilk and Fisher tests, respectively. Depending on the results, parametric or non-parametric tests were used. A significance level of $\alpha < 0.05$ was used for all tests. Values are means ± SD.

In univariate analyses, correlations between Hg and continuous explanatory variables (feather $\delta^{13}C$ and $\delta^{15}N$ values, and the duration of the chick rearing period) were tested using Pearson correlations. In multivariate analyses, the influence of species and continuous explanatory variables on feather Hg concentrations were investigated using General Linear Models (GLMs). Feather Hg concentrations were log transformed and the models were constructed with a normal distribution and an identity link function. Explanatory variables were defined as follows: species as a factor, and feather $\delta^{15}N$, $\delta^{13}C$ values, and the duration of the chick rearing period as covariates. The influence of the sampling year on Hg concentrations was not incorporated in model selection, since, as most species were sampled in only one year (Table 2), the year effect is confounded by the species effect. Noticeably however, two species (the Kerguelen petrel and subantarctic skua) that were sampled twice did not present

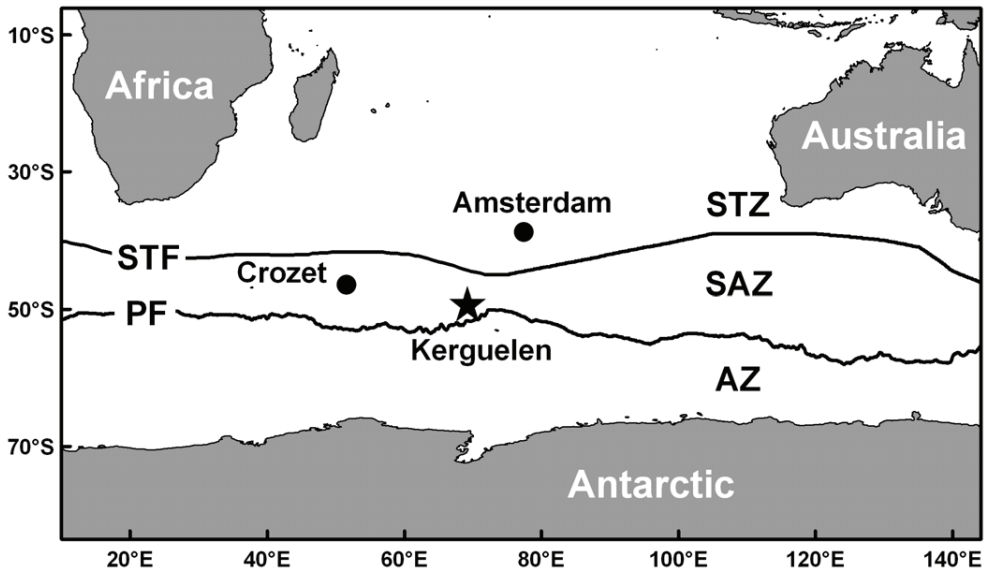

Figure 1. Location of the Kerguelen Islands and of the main oceanic fronts and zones in the southern Indian Ocean. Abbreviations: STF, Subtropical Front; PF, Polar Front; STZ, Subtropical Zone; SAZ, Subantarctic Zone; AZ, Antarctic Zone [23].
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significant inter-annual differences in feather Hg concentrations (data not shown).

Biologically relevant models were constructed by incorporating the different variables and their interactions. Continuous variables that were significantly correlated were not included in the same models. The most parsimonious models were selected according to the bias-adjusted Akaïke's Information Criterion (AICc), which is a small-sample bias adjustment [28,29]. As a general guideline, if AICc values differ by more than 2, the model with the lowest AICc value is the most accurate, whereas models with AICc values

Table 2. Chick feather Hg, $\delta^{13}C$ and $\delta^{15}N$ values of Kerguelen seabirds.

Species	Years of sampling	*n*	Total Hg ($\mu g \cdot g^{-1}$ dry mass)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
South Georgian diving petrel	2012	16	0.05±0.01 (0.04–0.08)	−21.3±0.3	8.8±0.3
Common diving petrel	2003	17	0.11±0.02 (0.07–0.15)	−17.0±0.5	12.1±0.4
Antarctic prion	2008	10	0.21±0.05 (0.16–0.31)	−21.5±0.5	9.3±0.4
Thin-billed prion	2003	9	0.22±0.09 (0.12–0.40)	−21.5±0.5	9.1±0.4
Southern rockhopper penguin	2007	12	0.27±0.06 (0.20–0.37)	−15.3±0.4	11.5±0.4
Macaroni penguin	2007	12	0.36±0.07 (0.25–0.52)	−18.3±0.5	10.0±0.5
Kelp gull	2011	7	0.73±0.38 (0.40–1.38)	−12.8±0.7	13.4±1.0
Kerguelen petrel	2009, 2010	18	0.78±0.17 (0.51–1.20)	−22.1±0.5	11.7±0.5
Blue petrel	2003	13	0.84±0.18 (0.58–1.14)	−21.8±0.5	9.8±0.5
King penguin	2006	12	1.12±0.16 (0.83–1.50)	−21.6±0.3	10.6±0.3
White-headed petrel	2003	10	1.54±0.34 (1.07–1.99)	−22.0±0.5	12.2±0.2
Great-winged petrel	2005	10	1.64±0.48 (0.96–2.68)	−20.0±0.4	12.9±0.4
White-chinned petrel	2005	14	1.82±0.51 (1.13–2.76)	−22.2±0.7	11.3±0.8
Kerguelen shag	2006	10	2.21±1.06 (1.35–4.64)	−13.8±1.0	14.0±0.6
Gentoo penguin	2006	12	2.45±0.67 (1.14–3.66)	−16.5±1.2	12.4±0.8
Light-mantled sooty albatross	2005	15	2.46±0.67 (1.56–3.69)	−21.0±0.4	12.6±0.4
Black-browed albatross	2005	18	2.58±0.59 (1.54–3.70)	−18.5±0.8	12.9±0.5
Grey petrel	2005	16	3.16±1.21 (1.59–5.70)	−19.9±0.6	13.6±0.4
Wandering albatross	2005	15	4.45±1.60 (2.19–8.43)	−19.3±0.4	14.2±0.4
Subantarctic skua	2005, 2010	22	5.15±1.56 (2.40–7.93)	−21.8±0.4	10.8±0.3
Northern giant petrel	2005	12	5.31±1.12 (4.06–7.94)	−19.2±1.2	13.4±0.8

Species were deliberately ranked by increasing Hg concentrations and not in taxonomic order. Values are means ± SD with ranges in parentheses for Hg.
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differing by less than 2 are fairly similar in their ability to describe the data, and the model including the least number of parameters (the simplest) is the most accurate [30]. The likelihood of a model, referred to as the Akaïke weight (w_i) was estimated following Johnson and Omland [31] (Table 3). The w_i can be interpreted as approximate probabilities that the model i is the best one for the observed data, given the candidate set of models. Model fit was assessed by a chi-square goodness-of-fit test [32], and residuals were checked for normality using Shapiro-Wilk test and Q-Q plot. The coefficient of determination R^2adj was calculated for each model [31] (Table 3).

Results

Univariate analysis showed that total Hg contamination varied widely within the seabird community, with chick feather Hg concentrations differing significantly between species (Kruskal-Wallis, $H = 260.23$, $P<0.001$, $n = 21$). Mean Hg concentrations ranged from 0.05 ± 0.01 to 5.31 ± 1.12 $\mu g \cdot g^{-1}$ dry mass in South-Georgian diving-petrels and northern giant petrels, respectively (Table 2). The lowest chick feather Hg concentration occurred in a common diving-petrel and the highest in a wandering albatross (0.04 and 8.43 $\mu g \cdot g^{-1}$, respectively).

No overall significant correlation between feather Hg and $\delta^{13}C$ values was found (Pearson correlation, $r = -0.08$, $t = -1.31$, $P = 0.19$, $n = 280$), while feather Hg concentration was highly significantly and positively correlated with $\delta^{15}N$ values ($r = 0.48$, $t = 9.19$, $P<0.001$). Feather Hg concentration was also significantly and positively related to the duration of the chick rearing period ($r = 0.26$, $t = 4.54$, $P<0.001$). Furthermore, feather $\delta^{15}N$ and $\delta^{13}C$ values were positively correlated ($r = 0.63$, $t = 10.24$, $P<0.001$), and they were significantly related to the duration of the chick rearing period ($r = -0.12$ and 0.35, $t = -2.10$ and 6.33, $p = 0.037$ and <0.001 for $\delta^{13}C$ and $\delta^{15}N$ values, respectively). When focusing on oceanic seabirds (12 species and 158 individuals) a highly significant and positive correlation was found between feather Hg and both $\delta^{13}C$ ($r = 0.61$, $t = 9.68$, $p<0.001$) and $\delta^{15}N$ values ($r = 0.80$, $t = 16.90$, $P<0.001$) (Fig. 2).

In multivariate analyses, the most parsimonious GLM model selected by AICc values included the effects of species and $\delta^{15}N$ (Table 3) in explaining Hg concentrations in body feathers. Indeed, Hg concentrations differed significantly among species ($F20,\ 258 = 217$, $P<0.001$) and were significantly and positively related to their $\delta^{15}N$ values ($F1,\ 278 = 2776$, $P<0.001$). The second-ranked model included an effect of species, $\delta^{13}C$ and their interaction, but it had a low likelihood when compared to the first-ranked model (Table 3). Models including the duration of the chick rearing period had also a low likelihood, thus explaining poorly feather Hg concentrations when compared to species and feather $\delta^{15}N$ values.

Table 3. AICc model ranking for feather Hg concentrations within the Kerguelen seabird community (see text for details).

Models	AICc	ΔAICc[a]	w_i[b]	R^2adj	*gdf*
species+$\delta^{15}N$	78.79	0.00	0.999	0.96	1.00
species+$\delta^{13}C$+species* $\delta^{13}C$	97.08	18.29	<0.001	0.96	1.00
species+$\delta^{15}N$+species* $\delta^{15}N$	99.36	20.57	<0.001	0.96	1.00
species+CRP+ species* CRP	110.12	31.33	<0.001	0.96	1.00
species+CRP	110.12	31.33	<0.001	0.96	1.00
species	110.12	31.33	<0.001	0.96	1.00
species+$\delta^{13}C$	110.20	31.41	<0.001	0.96	1.00
$\delta^{15}N$	841.16	762.37	<0.001	0.37	0.03
CRP	930.02	851.23	<0.001	0.14	<0.001
null	971.35	892.56	<0.001	0.00	<0.001
$\delta^{13}C$	972.11	893.32	<0.001	<0.01	<0.001

Abbreviations: AICc, bias-adjusted Akaike's Information Criteria values; w_i, AICc weights; R^2adj, R-squared adjusted; *gdf*, goodness-of-fit; CRP, duration of the chick rearing period.

[a]Scaled ΔAICc; ΔAICc = 0.00 is interpreted as the best fit to the data among the models.

[b]Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

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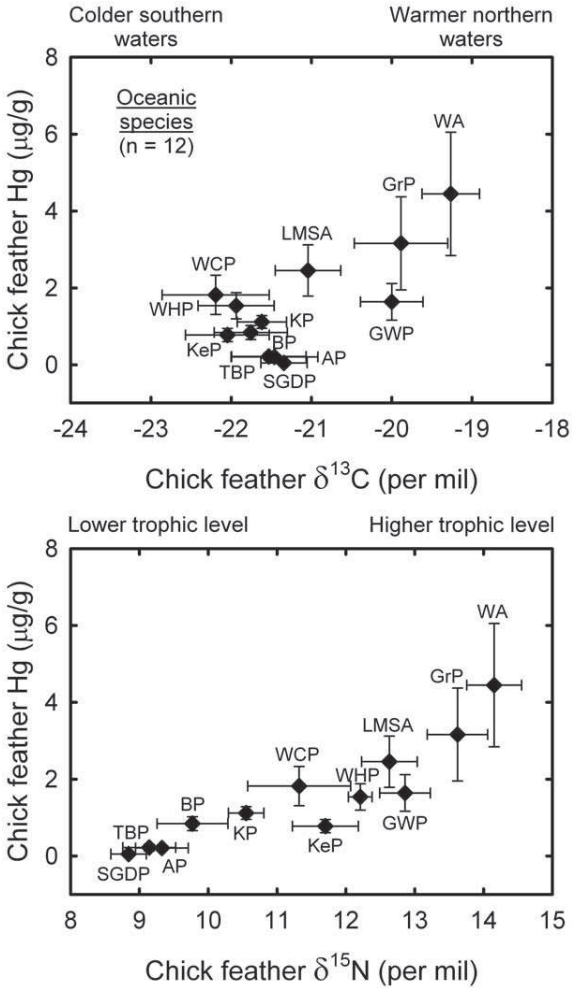

Figure 2. Relationship between chick feather Hg concentrations (means ± SD; $\mu g \cdot g^{-1}$ dry mass) and (a) foraging habitat (chick feather $\delta^{13}C$) and (b) trophic position (chick feather $\delta^{15}N$) of oceanic species. Filled diamonds and empty circles refer to oceanic and other species, respectively. See Table 1 for species abbreviations.

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Discussion

To the best of our knowledge, this study is the first to investigate Hg contamination in such a large number of sympatric seabirds. It completes the few works previously conducted in the southern Atlantic [33,34] and the southern Pacific Oceans [35,36], thus partly filling the gap of knowledge from the southern Indian Ocean sector [9]. The study adds a substantial number of seabird chicks that were either not previously investigated (n = 16) or inadequately sampled (n = 4) [35], with only data from wandering albatross [37] being available in the scientific literature (Table 4).

Statistical analyses pointed out the important effect of species, feeding habits ($\delta^{15}N$) and foraging habitats ($\delta^{13}C$) on chick feather Hg concentrations, which, by contrast, are little explained by the duration of the chick rearing period. However, univariate analysis showed a positive relationship between Hg contamination and the duration of the chick- rearing period, the most likely explanation being that assimilated Hg accumulates during chick growth over weeks and months and is ultimately excreted in newly grown feathers at the end of the period [18]. The positive co-variation between the duration of the chick rearing period and $\delta^{15}N$ likely results from the longer chick rearing period of large seabirds (*e.g.* albatrosses) that feed at higher trophic positions than smaller species with shorter growth period (*e.g.* petrels).

Feather Hg Concentrations: Comparison with Other Species and Areas

Kerguelen seabird chicks presented a wide range of Hg concentrations, with the highest contaminated species containing ~102 times (two orders of magnitude) more feather Hg than the less contaminated species. Both the lowest and highest Hg values occurred in flying birds, with contamination levels of the flightless penguins ranging from low to intermediate values. No other feather Hg concentrations are available from Kerguelen seabirds, but a preliminary analysis conducted on internal tissues of adults of five species of zooplankton-eating petrels [9] ranked the species in the same decreasing order than in the present study, with blue petrels containing more Hg than prions and diving petrels. In the same way, the decreasing order is roughly the same in the only other comparable investigations that were conducted on seabirds breeding in the southern Atlantic Ocean [33,37]. Feather Hg was higher in albatrosses and giant petrels, intermediate in the white-chinned petrel and lower in the blue petrel, prions and diving petrels. Hg concentrations were however much higher in south Atlantic Procellariiformes than in the present study [33], with the most likely explanation being that work was conducted on adult birds, not on chicks. Indeed, adult birds have consistently higher feather Hg concentrations than their chicks [38]. Adults have a longer period to assimilate and accumulate metals from their food between two successive moults, whereas chicks only have the several weeks (to months) of the chick-rearing period [39].

A review of the existing literature on seabird chicks (Table 4) shows that mean feather Hg concentration can reach very high values in acutely polluted areas (up to 36.4 $\mu g \cdot g^{-1}$ for common terns in the German North Sea). Elsewhere, however, Hg concentration ranges from 0.05 to 5.6 $\mu g \cdot g^{-1}$ (the sooty tern and black-footed albatross, respectively). This range is remarkably similar to that from the present investigation, indicating that seabirds from only one location (Kerguelen) encompass the whole range of values that were collected worldwide in poorly industrialized areas. Consequently, comparison of Hg levels between distant locations using seabirds as sentinels of environmental contamination necessitates collecting feathers from many species, because a small subset could not be fully representative of the whole seabird assemblage, and thus of the surrounding marine environment.

At the species level, chick feather Hg concentrations from Kerguelen seabirds fall within the concentration range reported for similar species elsewhere. The wandering albatross from South Georgia [37], presented identical Hg concentrations than chicks from Kerguelen, and the taxonomically closely-related but spatially distant Scottish great skua and Kerguelen subantarctic skua showed almost similar feather Hg concentrations, depending on breeding colonies (Table 4). At the community level, only two previous investigations on chick Hg contamination included more than five sympatric species and both were conducted in tropical waters of the Pacific (Midway Atoll, six species [40]) and the Indian Oceans (the Seychelles, seven species [38]). Based on feather Hg concentrations, the Kerguelen seabird community compares well with the Midway Atoll assemblage (0.34–5.57 $\mu g \cdot g^{-1}$) that also includes albatrosses, i.e. the species group that contains the highest feather Hg concentrations among seabirds [12] (Table 4). By contrast (and excluding albatross data), chick feather Hg concentrations were overall higher in Kerguelen species than in seabirds from the Seychelles (0.15–0.70 $\mu g \cdot g^{-1}$) and La Réunion (0.07–0.42 $\mu g \cdot g^{-1}$ [41]), thus suggesting higher Me-Hg bioavailability in subantarctic than in tropical waters of the Indian Ocean. The hypothesis remains to be investigated because there is a paucity of information on mercury speciations in marine waters, and more specifically, on the sources of Me-Hg to marine consumers, including seabirds and their prey [6,13].

Potential Adverse Effect

One of the key problems in environmental toxicology is to interpret the impact of observed contaminant concentrations. In general, Hg levels of 1.5–18 $\mu g \cdot g^{-1}$ dry mass in eggs are sufficient to cause decreased egg mass, embryo malformations, lower hatchability, decreased chick growth, and lowered chick survival [42]. The threshold levels in feathers of negative effects to chicks is currently unknown, but laboratory and field studies on adult birds indicate that feather Hg concentrations of 2.4 to 40 $\mu g \cdot g^{-1}$ are associated with adverse effects, with the commonest used toxicity threshold being 5 $\mu g \cdot g^{-1}$ [42,43]. Feather Hg concentrations of most Kerguelen seabirds are below this threshold, but some individuals from four species showed higher levels. Namely, 64%, 50%, 33% and 13% of chicks of subantarctic skuas, northern giant petrels, wandering albatrosses and grey petrels, respectively, exceeded the threshold value and were thus potentially threatened by Hg (Fig. 3). However, those levels are difficult to interpret because chicks showed no observed obvious ill effects. Moreover, seabirds cope efficiently with high Hg concentrations in their prey through efficient detoxification processes and, hence, they are expected to have higher toxicity thresholds than terrestrial birds [36]. Nonetheless, such comparisons are invaluable to use seabirds as sentinels of ecosystem health, because it provides ways to identify not only the species the more at risk, but also the species that would be useful as bio-indicators [10].

Feather Hg Concentrations, Diet and Trophic Levels

As expected, the overall statistical analysis indicated a $\delta^{15}N$ effect on feather Hg concentrations within the Kerguelen seabird community. The positive correlation verifies our hypothesis stating that Hg concentration should increase with trophic level, because $\delta^{15}N$ is a proxy of consumers' trophic position [44]. Noticeably, the relationship was partially hindered by pooling species that forage in distinct habitats (neritic vs. oceanic and benthic vs. pelagic) marked by different isotopic baselines. Within this context, the positive correlation between Hg and $\delta^{15}N$ is particularly

Table 4. An overall synthesis of Hg concentrations (means ± SD with ranges in parentheses; $\mu g \cdot g^{-1}$ dry mass) in body feathers of seabird chicks.

Species	Location	n	Total Hg	References
Spheniscidae				
Adelie penguin (*Pygoscelis adeliae*)	Terra Nova Bay (Antarctica)	11	0.37±0.15	[67]
Diomedeidae				
Wandering albatross (*Diomedea exulans*)	Bird Island (South Georgia)	10	3.31±0.68	[37]
Black-footed albatross (*Phoebastria nigripes*)	Midway Atoll	17	5.57±0.36[a]	[40,68]
Laysan albatross (*Phoebastria immutabilis*)	Midway Atoll	35	2.15±0.12[a]	[40,68]
		15	1.95±0.15[a]	[69]
Procellariidae				
Barau's petrel (*Pterodroma baraui*)	La Réunion	32	0.30±0.07	[41]
Bonin petrel (*Pterodroma hypoleuca*)	Midway Atoll	20	3.87±0.32[a]	[40,70]
Audubon's shearwater (*Puffinus lherminieri*)	La Réunion	38	0.07±0.01	[41]
	Aride Island (Seychelles)	10, 8	0.15±0.03, 0.27±0.06	[38]
Pink-footed shearwater (*Puffinus creatopus*)	Mocha Island (Chile)	8	0.36±0.10	[71]
Sooty shearwater (*Puffinus griseus*)	Humboldt Current (Peru)	14	0.72±0.35	[72]
	New Zealand Region	4	0.8±0.8	[35]
Wedge-tailed shearwater (*Puffinus pacificus*)	Aride Island (Seychelles)	10	0.39±0.05	[38]
Cory's shearwater (*Calonectris diomedea*)	Azores	14	0.87±0.10[a] (0.33–1.5)	[73]
		30	0.7±0.2	[74]
	Berlengas (Portugal)	25	1.1±0.3	[74]
Hydrobatidae				
Leach's storm-petrel (*Oceanodroma leucorhoa*)	New Brunswick (Canada)	20	1.42 (0.90–2.22)[b]	[75]
Phaethontidae				
Red-tailed tropicbird (*Phaethon rubricauda*)	Midway Atoll	12	2.51±0.28[a]	[40,70]
White-tailed tropicbird (*Phaethon lepturus*)	La Réunion	16	0.29±0.02	[41]
	Aride Island (Seychelles)	10, 10	0.52±0.11, 0.70±0.10	[38]
Phalacrocoracidae				
European shag (*Phalacrocorax aristotelis*)	Atlantic sector (Spain)	20, 12	from 0.54±0.19 to 1.07±0.38	[76]
	Cantabrian sector (Spain)	15, 10	3.09±1.40, 5.09±1.82	[76]
Stercorariidae				
Great skua (*Catharacta skua*)	Foula (Shetland)	40	1.3±0.4	[54]
		28	1.22±0.38	[77]
		29	2.16±1.15	[53]
	St Kilda (Outer Hebrides)	22	5.37±1.29	[53]
Arctic skua (*Stercorarius parasiticus*)	Foula (Shetland)	30	0.46±0.22	[77]
Laridae				
Audoin's gull (*Larus audouinii*)	Dodecanese (Greece)	20, 10	from 0.94±0.27 (0.61–1.46) to 1.71±0.48 (0.32–2.55)	[78]
	Cyclade (Greece)	20, 10	from 1.42±0.29 (0.88–2.04) to 2.02±0.38 (1.13–2.45)	[78]
	Kythera (Greece)	8	1.20±0.33 (0.76–1.77)	[78]
	Ebro Delta (Western Mediterranean)	39	5.09 (4.68–5.54)[d]	[79]
	Alboran Island (Western Mediterranean)	15	3.87 (3.28–4.57)[d]	[79]
	Chafarinas Islands (Western Mediterranean)	12	3.17 (2.31–4.35)[d]	[79]
Black-headed gull (*Larus ridibundus*)	German North Sea	36	0.88±0.53 (0.14–2.11), 0.94±0.45 (0.10–2.07)	[80]
Common gull (*Larus canus*)	Elbe estuary (German North Sea)	12	2.24±1.84	[81]
	Jade Bay (German North Sea)	11	1.40±0.37	[81]
Franklin's gull (Larus pipixcan)	Interior U.S.A.	≥79	0.80±0.06a	[82]
	Minnesota	15	0.31±0.11a	[83]

Table 4. Cont.

Species	Location	n	Total Hg	References
Glaucous-winged gull (*Larus glaucescens*)	Aleutian Islands	36	1.98±0.18[a]	[84]
Herring gull (*Larus argentatus*)	New York	15, 20, 15	0.81±0.12, 1.80±0.11, 2.83±0.27[a]	[85,86]
	New Jersey	14, 15	1.76±0.35, 2.58±0.23[a]	[86]
	Virginia	15	0.76±0.11[a]	[86]
	German North Sea	38	5.88±4.90 (0.78–27.14)	[87,88]
		39	1.27±0.60 (0.47–2.98), 1.31±0.62 (0.49–2.89)	[80]
	Shetland	12	2.24±0.83 (1.04–4.12)	[88]
Red-billed gull (*Larus novaehollandiae*)	Kaikoura Peninsula (New Zealand)	27	2.02±1.16	[89]
Yellow-legged gull (*Larus michahellis atlantis*)	Azores	34	2.3±1.0	[74]
	Madeira	22	2.6±0.8	[74]
	Berlengas (Portugal)	28	2.4±0.5	[74]
Kittiwake (*Rissa tridactyla*)	Foula (Shetland)	26	0.37±0.12	[77]
	Shetland	9	0.49±0.28 (0.26–1.03)	[88]
	German North Sea	13	2.65±0.61 (1.61–3.64)	[88]
	Northeast Norway	27	0.55±0.10	[89]
Sternidae				
Arctic tern (*Sterna paradisaea*)	Foula (Shetland)	15	0.69±0.14	[77]
Common tern (*Sterna hirundo*)	Bird island (Massachusetts)	21	3.1±0.2[a]	[90]
		15	4.2±3.1	[91]
	Long Island (New York)	16	1.4±0.6 (0.6–2.6)	[92]
		14, 21	2.01±0.25, 2.61±2.55[a]	[93]
	German North Sea	21	6.14±4.33 (1.51–18.40)	[87]
		13	3.00±0.50 (1.97–3.74), 3.26±0.70 (2.41–4.91)	[80]
		27	12.89±6.90 (1.51–70.00)	[88]
	Elbe estuary (German North Sea)	4	36.4±18.9 (21.7–62.9)	[88]
	Jadebusen (German North Sea)	9	3.8±0.7 (2.9–5.1)	[88]
	East Scotland	19	1.80±0.79 (0.92–3.11)	[88]
	Shetland	12	1.40±0.72 (0.84–2.95)	[88]
	Azores	10, 19	from 1.1±0.4 to 1.5±0.4	[74]
Forster's tern (*Sterna forsteri*)	San Francisco Bay	89	6.44±0.28[d]	[94]
Little tern (*Sterna albifrons*)	Portugal	168	4.07±1.42	[95]
	Vaia (Portugal)	12, 10	4.40±1.31, 4.67±1.38	[96]
Roseate tern (*Sterna dougallii*)	Azores	19, 14	0.8±0.2, 1.1±0.2	[74]
	Aride Island (Seychelles)	12	0.69±0.32	[97]
White tern (*Gygis alba*)	Aride Island (Seychelles)	10, 10	0.21±0.03, 0.40±0.05	[38]
	Midway Atoll	7	1.65±0.18[a]	[40,98]
Sooty tern (*Onychoprion fuscata*)	Lys (Glorieuses)	32	0.05±0.03	[41]
	Aride Island (Seychelles)	10	0.26±0.05	[38]
	Hawaii	16	0.16±0.02[a]	[98]
Brown noddy (*Anous stolidus*)	Aride Island (Seychelles)	10, 10	0.27±0.05, 0.37±0.06	[38]
	Hawaii	20	0.07±0.003[a]	[99,100]
Lesser noddy (*Anous tenuirostris*)	Aride Island (Seychelles)	10, 5	0.17±0.03, 0.41±0.17	[38]
Alcidae				
Razorbill (*Alca torda*)	New Brunswick (Canada)	16	1.40 (0.86–2.29)	[75]
Common murre (*Uria aalge*)	New Brunswick (Canada)	9	1.14 (0.59–2.18)[b]	[75]
Atlantic puffin (*Fratercula arctica*)	New Brunswick (Canada)	17	1.00 (0.27–0.73)[b]	[75]

Down Hg values and studies with too low numbers of sampled chicks (n <4) were excluded.
[a]Values are means ± SE.
[b]Values are estimated marginal means with 95% confidence limits in parentheses.
[c]Median value.
[d]Values are geometric means with 95% confidence limits in parentheses.
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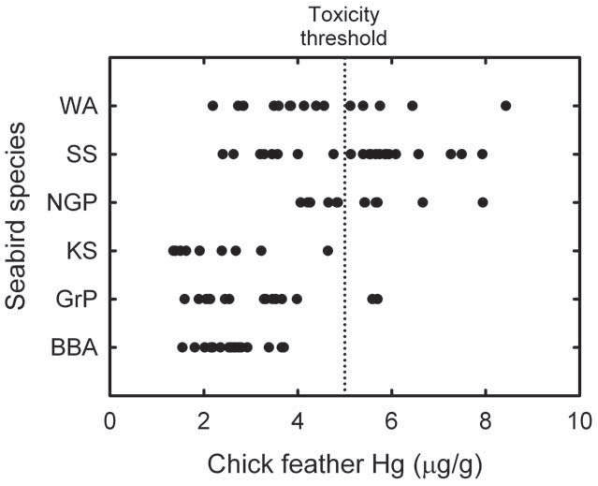

Figure 3. Feather Hg concentrations ($\mu g \cdot g^{-1}$ dry mass) of individual chicks from the six most contaminated species from the Kerguelen seabird community. See text for toxicity threshold and Table 1 for species abbreviations. BBA illustrates the most contaminated species of the assemblage with all individual values being below the threshold value.
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relevant. Indeed, the relationship was even stronger when looking at oceanic seabirds only (Fig. 2). The feather $\delta^{15}N$ values of the 12 oceanic species ranged from 8.8‰ (the crustacean-eater South Georgian diving petrel) to 14.2‰ (the squid-eater wandering albatross), which, assuming a trophic enrichment factor of 2.7‰ for carnivorous organisms [45], corresponds to ~3 trophic levels. The corresponding feather Hg values indicated an 86-fold Hg enrichment within the oceanic seabird assemblage. Accordingly, Hg contents of pelagic organisms from the Southern Ocean (including Kerguelen Islands) increase in the order crustaceans <fish ≤ squids < seabirds [9,33,46]. Hence, while patterns of Hg accumulation in food webs of the open ocean are largely unknown [6], the present work highlights the occurrence of efficient Hg biomagnification processes in subantarctic waters of the southern Indian Ocean that merit further oceanographic investigations.

The subantarctic skua was clearly an outlier species within the Kerguelen seabird assemblage, with chick Hg concentration being high when compared to feather $\delta^{15}N$, and, to a lesser extent, $\delta^{13}C$ values (Table 2). At the study site, adult skuas forage on land where they feed their chicks almost exclusively with small seabirds, mainly blue petrels [47]. Feather $\delta^{15}N$ value of skua chicks agrees with a blue petrel-based diet, being 2.7‰ ^{15}N-enriched when compared to blood of their prey (author's unpublished data). The skua Hg content is also in agreement with feeding on blue petrels, because petrel muscle contains disproportionately more total Hg than fish and crustaceans [9,46]. High petrel Hg contamination is likely to result from the combination of two life-history traits of the species: (i) blue petrels are long-lived animals [48] and are thus prone to Hg bioaccumulation over the long-term, and (ii) they prey on crustaceans and mesopelagic fish [49,50], with the latter containing high Hg concentrations [46,51]. The trophic explanation of the high Hg levels of subantarctic skua chicks therefore suggests that seabirds feeding on other seabirds are at risk to accumulate critical pollutant loads. Indeed, Scottish great skuas feeding predominantly on other seabirds contain more Hg than those feeding on fish [52,53], but with no negative effects on breeding performance and survival [54].

Feather Hg Concentrations and Foraging Habitats

Univariate analysis indicated no $\delta^{13}C$ effects on feather Hg concentrations within the Kerguelen seabird community, and, in multivariate analysis, species and $\delta^{15}N$ produced the best model. This result seems to contradict our hypothesis that foraging habitat should play an important role in shaping seabird Hg contamination. However, the large range of seabird $\delta^{13}C$ values indicates that, again, species that forage within different isoscapes were pooled, thus resulting in a confounding effect [15] for the interpretation of feather Hg concentrations. For example, the five seabirds with $\delta^{13}C$ values ≥ -18‰ were all neritic species feeding either along the shoreline (the kelp gull) or on pelagic (the southern rockhopper penguin and common diving petrel) or benthic prey (the gentoo penguin and Kerguelen shag) (Table 1). The relatively high feather Hg concentrations in feathers of the two latter species are in agreement with the Me-Hg-enrichment of coastal benthic areas [6]. Noticeably, the two species feeding in the closed sea (the southern rockhopper penguin and common diving petrel) and the sibling species foraging in the open sea (the macaroni penguin and South Georgian diving petrel, respectively) have low feather Hg concentrations. Such low levels can be related to a crustacean-based diet and suggest that no significant inshore/offshore gradient occurs in the availability of Me-Hg in pelagic waters surrounding the Kerguelen Islands.

At a larger spatial scale, the importance of foraging habitat is exemplified by the positive correlation between feather Hg concentrations and $\delta^{13}C$ values in oceanic seabirds. Taken into account the latitudinal $\delta^{13}C$ gradient within the Southern Ocean [15,16], the relationship indicates that species foraging in cold waters south of Kerguelen Islands are less prone to be contaminated than species feeding in northern warmer waters. This finding does not fit well with the only study on Hg speciation in the Southern Ocean showing higher Me-Hg concentrations in Antarctic than in subantarctic and subtropical waters [13]. This mismatch reinforces the need to better document the bioavailability of Me-Hg in the main oceanic and neritic water masses and to determine the levels of Hg contamination of seabirds breeding in the north (e.g. Amsterdam Islands) and south (e.g. Adélie Land) of the Kerguelen Archipelago to confirm or not this latitudinal trend both at the bottom and at the top of the marine trophic webs.

Conclusions

The pattern of Hg contamination of Kerguelen seabirds is remarkable due to its wide range of values, but the circumpolar annular structure of the Southern Ocean [55] suggests it may be generalized to other subantarctic localities. The source of Hg in subantarctic waters is still poorly known, but it ultimately derives mostly from anthropogenic contamination, because (i) human activities have increased emissions to the atmosphere by approximately a factor of 3, and (ii) atmospheric deposition is the dominant input term in the world ocean [6], including the Southern Ocean [13]. Since global Hg emissions will increase in the future, Hg contamination will increase as well in remote areas [56]. Hence, Kerguelen Archipelago, together with other isolated islands located far away from anthropogenic sources, can be considered as ideal study sites to monitor the temporal trend of global Hg contamination. Our study allows selecting chicks of some seabirds as sentinels of environmental pollution according to their high Hg concentrations with relatively low variances and to their contrasted foraging ecology. Three representative species are the gentoo penguin (benthic neritic forager), black-browed albatross (pelagic neritic forager) and light-mantled sooty albatross (southern oceanic forager). Despite its larger variance in Hg

concentrations, the wandering albatross (northern oceanic forager) must also be included, because this iconic seabird is known to be among the most Hg contaminated vertebrate species [36,57].

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Author Contributions

Conceived and designed the experiments: YC P. Bustamante OC. Performed the experiments: P. Blévin AJ. Analyzed the data: P. Blévin AC. Contributed reagents/materials/analysis tools: P. Bustamante. Wrote the paper: YC P. Blévin.

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Paper 6

Trace elements and persistent organic pollutants in the tissues of Antarctic prions (*Pachyptila desolata*) at Kerguelen archipelago, southern Indian Ocean

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Abstract Trace elements and persistent organic pollutants (POPs) concentrations were investigated in blood, liver, kidney, muscle and feathers of 10 Antarctic prions (*Pachyptila desolata*) from Kerguelen Islands, southern Indian Ocean. This study aimed to assess precisely the concentrations, tissue distribution, and inter-tissue and inter-contaminant relationships in a seabird from the southern Indian Ocean. Liver, kidney and feathers presented the highest concentrations of arsenic (As), cadmium (Cd) and mercury (Hg), respectively. Concentrations of Cd, cuivre (Cu), iron (Fe), and zinc (Zn) correlated in liver and muscle, suggesting that uptake and pathways of metabolism and storage were similar for these elements. The major persistent organic pollutants (POPs)were PCB-153, PCB-138, 4,4'-DDE and Mirex. The concentration and tissue distribution patterns of trace elements and POPs were in accordance with previous results in related seabirds. Conversely, Antarctic prions showed surprisingly high concentrations of an emerging-POP, PBDE-209. This was rarely observed in seabirds and could be due to feeding habits or biotransformation capacities of this species. Overall, this study shows that even zooplankton-eating species are exposed to a wide range of environmental contaminants in the remote southern Indian Ocean, in particular emerging-POPs, which merits further toxicological investigation.

Keywords: seabirds · trace elements · POPs · Southern Ocean · Procellariiformes · tissue distribution

INTRODUCTION

Trace elements and persistent organic pollutants (POPs) are commonly found in terrestrial and aquatic ecosystems worldwide (Jones and de Voogt, 1999). Trace elements and POPs, which come from both natural and anthropogenic sources, exhibit toxic properties (AMAP, 2004) causing endocrine dysfunction, mutagenesis, or reproductive and behavioural disturbances (e.g., Tanabe, 2002; Scheuhammer, 1987). Aquatic environments, including marine ecosystems, are major repositories of contaminants. Hence, marine organisms are particularly exposed to contaminants from anthropogenic and natural sources, and exposure is governed by various factors such as trophic position and foraging habitat (Anderson et al., 2010).

Seabirds are integral components of aquatic ecosystems; they are long-lived, forage over large geographic areas and feed at different trophic levels. Therefore, they are often considered to be ideal models to biomonitor contaminants from the marine environment such as trace elements or POPs (Furness and Camphuysen, 1997). Several bird tissues have been promoted for monitoring avian exposure, particularly feathers (e.g., Seco Pon et al., 2011; Bustnes et al., 2002), blood (e.g., González-Solís et al., 2002; Bustnes et al., 2007) and liver (e.g., Jerez et al., 2013; Colabuono et al., 2012). Yet there have been only few comprehensive studies that have simultaneously quantified POPs and trace elements in soft tissues, feathers and blood, and that studied the relationships among a suite of tissues and between contaminants (Eagles-Smith et al., 2008).

Although the Southern Ocean is a remote environment, away from emission sources of contaminants, it is subjected to trace element and POP contaminations due to global transport through atmospheric and oceanic circulation (Gaiero et al., 2003; Wania and MacKay, 1996; Bargagli, 2008). Previous studies have examined the concentrations of these contaminants in various species in the Southern Ocean and Antarctica (e.g., Lock et al., 1992; Anderson et al.,

2010; Corsolini et al., 2002; van den Brink et al., 1998), especially Procellariiformes which feed in a wide range of ecological niches. However, most studies on these compounds in subantarctic seabirds were carried out in the southern Atlantic and Pacific Oceans, whereas data on the contamination of seabirds in the southern Indian Ocean are still scarce (Bocher et al., 2003; Blévin et al., 2013; Carravieri et al., 2013; Scheifler et al., 2005).

In this context, the first objective of the present study was to investigate concentrations of trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn) and POPs (polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs)) in various tissues of the Antarctic prion (*Pachyptila desolata*) from the Kerguelen Islands, a remote subantarctic archipelago in the southern Indian Ocean. The Antarctic prion is a zooplankton feeder breeding in Antarctic and subantarctic islands, with important populations at South Georgia, Auckland and Kerguelen Islands (Marchand and Higgins, 1990; Weimerskirch et al., 1989; Cherel et al., 2002). In the present study, contaminant concentrations were quantified in blood, liver, kidney, muscle and feathers of birds. Bocher et al. (2003) examined trace elements in liver, kidney and muscle of zooplankton-eating species from the southern Indian Ocean, including the Antarctic prion, but these authors focussed exclusively on Cd, Cu, Hg and Zn. Moreover, except in subcutaneous fat and muscle of albatrosses from southern-east Indian Ocean (Guruge et al., 2001a, b; Tanabe et al., 2004), there are no data on POP concentrations in seabirds from this area. Thus, knowledge on seabirds' contamination in this part of the Southern Ocean is limited and need to be completed. Hence, this first descriptive step allows assessing contamination levels of the poorly known southern Indian Ocean marine ecosystem.

The second objective of the study was to investigate the tissue distribution of these contaminants, and inter-tissue and inter-contaminant relationships. The monitoring of contaminant concentrations and the study of their possible effects depends on the tissue

analysed (Henriksen et al., 1998). As noted by previous studies (e.g., García-Fernández et al., 2013; Burger, 1993), the use of blood and feathers samples has several advantages, especially for endangered species. Importantly, non-destructive sampling allows long-term studies with repeated sampling on the same birds and therefore the monitoring of potential impact of contaminants on demographic parameters (Erikstad et al., 2013; Goutte et al., 2014). Hence, a correlation of compounds between soft tissues, blood and feathers could validate the use of blood and feathers samples as probe for contaminants burden for the Antarctic prion, confirming previous studies on various polar seabirds (Henriksen et al., 1998; Bustnes et al., 2003). In seabirds, once ingested, elements are transported in the blood, deposited in various tissues, and excreted or stored. Concentrations in blood samples mainly reflect short-term exposure through the diet (Burger and Gochfeld, 1997). Concerning soft tissues, liver and kidney are specifically involved in the detoxification of contaminants, while muscles could function as a storage tissue (Lewis and Furness, 1991). Lastly, birds' feathers sequester trace elements during their growth (Burger, 1993). At least, feathers are the major pathway for mercury excretion (Braune and Gaskin, 1987; Monteiro and Furness, 1995). Therefore, a strong relationship between contaminant concentrations within each tissue may suggest common uptake and storage pathways, or similar regulation and detoxification processes (Mendes et al., 2008).

MATERIAL AND METHODS

2.1. Sample collection and preparation

Ten recently dead Antarctic prions trapped in the vegetation (*Acaena adscendens*) were opportunistically collected on January 26th, 2012, on the Kerguelen archipelago (49°21'S, 70°18'E), southern Indian Ocean. Only intact specimens were collected and then stored at -20°C until dissection. Age and breeding status of birds were not known. However, because in

Kerguelen Islands Antarctic prions' eggs are laid in December (incubation of the single white egg takes 44-46 days) and chicks fledge at 45-55 days old (Weimerskirch et al., 1989) these birds cannot be newly fledged chicks.

During necropsies, internal tissues (liver, kidneys and pectoral muscle) were sampled, weighted and wrapped individually in plastic bags and in aluminium foils for trace element and POP analyses, respectively. Clotted blood was collected from heart auricles and stored in microtubes at -20°C. Four body feathers were pulled out and stored dry in plastic bags. Birds were first sexed during necropsies by visual gonads examination. Sex was then confirmed using the molecular method described by Fridolfsson and Ellegren (1999). Prior to chemical analyses, internal tissues and blood were freeze-dried, ground to powder and then stored in plastic and glass tubes for trace element and POP analyses, respectively. Feathers were washed to remove surface dirt and adsorbed contaminants in a chloroform-methanol solution and then oven dried as described by Carravieri et al. (2013). For each individual, the four feathers were pooled and homogenized by cutting them with scissors into small fragments. Samples were weighed before and after freeze-drying to calculate water content (moisture).

2.2. Analyses of trace elements

Trace elements were determined in blood, liver, kidney, muscle and feathers. Total Hg analysis was carried out with an advanced mercury analyser (ALTEC AMA 254) on dried tissue aliquots (2-4 mg) following Blévin et al. (2013). All analyses were repeated 2–3 times until having a relative standard deviation <10%. Accuracy was checked using TORT-2 Lobster Hepatopancreas (NRC, Canada) as certified reference material (CRM) with a certified Hg concentration of 0.27 ± 0.06 μg g^{-1} dry weight (dw). Our measured values were 0.267 ± 0.006 μg g^{-1} dry mass (n = 18). Twelve other elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn) were analysed using a Varian Vista-Pro ICP-OES and a Thermo Fisher

Scientific X Series 2 ICP-MS (following Métian et al., 2008). Aliquots of the biological samples (30–300 mg) were digested with 6 ml 67–70% HNO_3 and 2 ml 34–37% HCl (Fisher Scientific, trace element grade quality), except for feathers (1.8 ml HNO_3 and 0.6 ml HCl). Acidic digestion was carried out overnight at room temperature and then in a Milestone microwave (30 min with constantly increasing temperature up to 120°C, and finally 15 min at this maximal temperature). Each sample was completed to 50 ml (15 ml for feathers) with milli-Q water. Three control samples (two Certified Reference Materials, and one blank) treated and analysed in the same way as the samples were included in each analytical batch. CRMs were DOLT-4 dogfish liver (NRC, Canada) and TORT-2 (NRC, Canada). The results were in good agreement with the certified values, with a mean recovery rate of 87-104% for DOLT-4 and 88-102% for TORT-2. Trace element concentrations are expressed in $\mu g\ g^{-1}$ dw.

2.3. Analyses of persistent organic pollutants (POPs)

POPs were analyzed in liver, kidney and muscle. The seven congeners considered as indicators PCBs (CBs 28, 52, 101, 118, 153, 138, and 180) were targeted. These compounds are predominantly present in biotic and abiotic matrices and were thus recognized as compounds representative of the whole group of PCBs by the Agency for Toxic Substances and Disease Registry (ATSDR, 2000). Additionally, 11 OCPs (HCB, γ-HCH, heptachlor, cis-chlordane, trans-nonachlor, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT and Mirex) and 12 PBDEs (BDEs 17, 28, 49, 71, 47, 66, 100, 99, 154, 153, 183 and 209) were also assayed. PCB and OCP standard solutions were provided by NIST (via LGC Standards, Molsheim, France) while PBDE standards were provided by Wellington Laboratories (via BCP Instruments, Irigny, France). Analytes were extracted using microwave assisted extraction (Milestone Start-E) with 10 mL of dichloromethane on homogenized freeze-dried samples (0.2-1.0g) spiked with internal standards: CBs 30, 103, 155 and 198, F-

BDE-47 (Chiron, via BCP Instruments), BDE-181 (Wellington Laboratories) and BB-209 (LGC Standards, Molsheim, France) (5-8 ng each) (Müller et al., 2001; Tapie et al., 2008). Extracts were re-concentrated into 300 µl of isooctane, using a RapidVap vacuum evaporation system from Labconco (Kansas City, MO, USA) and a nitrogen flow, prior to purification on acid silica gel column. PCBs, OCPs and PBDEs were eluted with 3-5 mL of pentane/dichloromethane (90/10; v/v), and final extracts were concentrated and transferred into isooctane as solvent keeper. Octachloronaphthalene (1 ng) was added as performance standard to quantify internal standards. Lipids were determined by gravimetry after filtration and evaporation of an aliquot of the DCM extract. PCBs and OCPs analyses were carried out on an HP 5890 series II gas chromatograph from Hewlett-Packard (Avondale, CA, USA) coupled to a ^{63}Ni electron capture detector (ECD). A capillary column HP5-MS (Agilent Technologies, Massy, France) was used (30 m x 0.25 mm x 0.25 µm). Helium (He, 5.6 quality, Linde Gas, Toulouse, France) was used as carrier gas at a flow rate of 1 ml min^{-1} and nitrogen (N_2, 5.0 quality, Linde Gas, Toulouse, France) was used as make up gas (60 ml min^{-1}). The injector temperature was 280°C and detector temperature was 320°C (Tapie et al., 2011).

PBDEs were analysed by gas chromatography coupled with mass spectrometry operated in negative chemical ionisation (GC-NCI-MS). Analyses were carried out using an Agilent 6890N GC coupled to a Quattro Micro GC (Waters Micromass). The system was fitted with J&W HP-5MS analytical column (15 m, 0.25 mm ID x 0.25 µm film thickness; Agilent Technologies, Massy, France) and operated in pulsed splitless injection mode (1.7 bar, 3 min) with an injector temperature of 280 °C. The helium carrier gas flow rate was 1.8 mL min^{-1} and temperature program was as follows: 90ºC (0.1 min), 185ºC (25°C min^{-1}), 275 °C (15 °C min^{-1}), and 305°C (35°C min^{-1}, held for 2 min). The transfer line temperature and the source temperature were set at 300 °C and 250°C, respectively. Ions were monitored in

SIM mode using a single acquisition window, with a dwell time set at 50 ms. $[Br]^-$ (m/z 79 and 81) was monitored for all PBDEs while m/z 402 and 404 were monitored for OCN.

For both analyses, PCBs, OCPs and PBDEs compounds were quantified relative to internal standards. CBs 30, 103, 155 and 198 were used to quantify PCBs and DDTd8 was used to quantify OCPs whereas F-BDE-47, BDE-181 and BB-209 were used to quantify PBDEs. A syringe standard (octachloronaphtalene) was used to quantify internal standards and verify recoveries for each sample. Quality control consisted of the analysis of solvent procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns, and analysis of certified reference material SRM 1947 (National Institute of Standards and Technology, USA) for PCBs, OCPs (except for γ-HCH, heptachlor and cis-chlordane), and PBDEs (except for BDEs 17, 18, 49, 71, 183 and 209). Procedure details are given in Tapie et al. (2008). As described by Labadie et al. (2010), POP concentrations were blank corrected and the method detection limit (LoD) was derived from the blank value variability, and for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of three. Regardless of the approach used for LoD calculation, the limit of quantification (LoQ) was set at three times LoD for all analytes.

2.6. Statistical analyses

Contaminants for which the concentration was lower than the limit of quantification (LoQ) in more than 30% individuals, for a particular tissue, were included in summary statistics but excluded from subsequent statistical analyses (Anderson et al., 2010; Borgå et al., 2006). Therefore, contaminants included in statistical analyses were 10 trace elements (As except in feathers, Cd, Cr except in blood, Cu, Fe, Hg, Mn, Ni except in blood and feathers, Se and Zn), 4 PCBs (CBs 118, 138, 153 and 180), 9 OCPs (HCB, γ-HCH and 2,4'-DDE except in kidney, cis-chlordane, trans-nonachlor, 4,4'-DDE, 4,4'-DDD and 4,4'-DDT only in muscle, and

Mirex), and only 2 PBDEs (BDE-28 except in kidney, and BDE-183 only in muscle). For these contaminants, concentrations below the LoQ and the detection limit (LoD) were replaced by LoQ*0.5 and LoD*0.5, respectively, and considered for statistical analyses (Anderson et al., 2010). Statistical tests were performed using R 2.15.1 (R Core Team, 2012) mainly following Crawley (2007). All data were first checked for normality and homogeneity of variances by means of Shapiro-Wilk and Bartlett tests, respectively. Most of the time these assumptions were not achieved, non-parametric analysis of variance was thus applied to assess differences in contaminant concentrations between tissues or gender (Wilcoxon test). Relationships between contaminant concentrations within and between tissues were tested using Spearman correlation rank test. Statistical significance of correlation coefficients was evaluated by using a bootstrap estimation method (Hall, 1992). Concentrations are presented as mean ± standard deviation (SD) in μg g^{-1} or ng g^{-1} dry weight.

RESULTS

3.1. Influence of sex in trace element and POP concentration

The 10 individuals of Antarctic prions included five females and five males. No consistent gender differences were found in tissue concentrations of trace elements and POPs. Hence, data from female and male birds were pooled for subsequent statistical analyses. The few significant differences included (i) higher As and Cd concentrations in feathers (Wilcoxon test, $p < 0.05$), and (ii) lower Hg concentrations in blood ($p < 0.01$), kidney ($p < 0.05$) and muscle ($p < 0.05$) of females than males.

3.2. Tissue distribution of trace elements

Concentrations of the 14 trace elements in blood, liver, kidney, muscle and feathers of Antarctic prions are listed in Table 1. Ag, Pb and V concentrations were below the detection

limits in all tissues, except Pb in feather samples. Similarly, concentrations of Co and Ni in blood and feathers and of Cr in blood were below the detection limits.

Trace elements showed different tissue distributions. Kidney and feathers presented the highest concentrations of the non-essential elements Cd and Hg, respectively (Wilcoxon test, $p < 0.01$) (Fig. 1). Liver presented the highest concentrations of the essential elements As, Fe, Mn and Zn ($p < 0.05$), muscle had the highest amount of Cu ($p < 0.001$) and blood showed the highest amount of Se ($p < 0.05$). In contrast, feathers presented the lowest concentrations of Fe and Se ($p < 0.001$) and blood the lowest concentrations of Cu, Mn, and Zn ($p < 0.01$).

3.3. Inter-tissue and inter- trace element relationships

Between-tissue relationships were investigated for each element. Only 11 correlations were significant out of 140, of which 6 were between blood and soft tissues. Significant positive relationships were found for Hg between blood and all soft tissues (Spearman test, $p < 0.05$), and between muscle and liver and kidney (Fig. 2). Significant positive correlations were also found for Cr between liver and muscle ($p < 0.05$), and for As between blood and kidney ($p < 0.01$).

Between-element relationships were investigated within each tissue. Most significant relationships were detected in blood and muscle. Correlations were particularly strong between Cr and Ni concentrations in liver, kidney and muscle ($rho \geq 0.9$, $p < 0.01$). With regard to essential elements, most significant relationships involved Cu, Fe, Mn and Zn that were strongly correlated in blood, liver and muscle. Blood presented the highest number of significant relationships between essential and non-essential elements (As-Cd, Cd-Cu, Cd-Fe, Cd-Mn and Cd-Zn), followed by muscle (Cd-Cu, Cd-Fe, Cd-Mn and Cd-Zn). In all tissues Cd was involved in the majority of relationships between essential and non-essential elements,

while no relationship between Hg and any essential element was detected. When considering non-essential trace elements, there were only significant relationships between Cd and Hg in liver.

3.4. Concentrations and tissue distribution of POPs

Mean concentrations of the 32 POPs (PCBs, OCPs and PBDEs) in liver, kidney and muscle of Antarctic prions are listed in Table 2. The following POPs were below the detection limit in all samples: 2,4'-DDD, Heptachlor (except in muscle), BDE-17, BDE-49+71, BDE-66, BDE-100 and BDE-99 (only in liver).

In all tissues, the PCB pattern was dominated by CB-153 > CB-138 > CB-118 which, all together, accounted for more than 75% of the PCBs burden. Prevalent OCPs compounds were 4,4'-DDE > Mirex > HCB, which accounted for more than 90% of the OCPs burden. BDE-209 presented the highest values amongst PBDEs, with only three individuals, however, showing concentrations above the quantification limit (LoQ = 1.0 ng g^{-1}).

Concentrations of PCBs and PBDEs in muscle, liver and kidney were generally not significantly different between tissues. In contrast, OCPs concentrations varied among tissues (Fig. 3). Namely, concentrations of γ-HCH, 2,4'-DDE, cis-chlordane and trans-nonachlor were higher in liver and muscle than in kidney (Wilcoxon test, $p < 0.05$), and concentrations of HCB ($p < 0.001$) were higher in liver and kidney than in muscle.

3.5. Relationships among tissues and between POPs

Relationships of POP concentrations between liver, kidney and muscle were determined using the sum (∑) of the different compounds. All between-tissue correlations were significant for ∑POPs, ∑PCBs and ∑OCPs (Spearman test, rho > 0.7, $p < 0.05$), and also for several individual PCBs and OCPs compounds (4,4'-DDE, Mirex, CB-138, CB-153 and CB-180, rho

> 0.7, $p < 0.01$). These compounds showed a particularly high degree of correlation between liver and kidney with rho > 0.88 ($p < 0.01$) (Fig. 4). Significant correlation between liver and kidney were also found for HCB, cis-chlordane and CB-118 (rho > 0.65, $p < 0.05$).

Relationships between POP concentrations within muscle, liver and kidney were also determined. ∑PCBs and ∑OCPs were significantly correlated in muscle, kidney and liver (respectively rho = 0.87, rho = 0.89, rho = 0.84, $p < 0.01$). A correlation matrix performed on each POP category showed that most of significant relationships were found within two groups of POPs. In the first group, there were strong correlations between HCB, trans-nonachlor, 4,4'-DDE, Mirex, CB-138, CB-153 and CB-180 in liver, kidney and muscle (Fig. 5). Additionally, 4,4'-DDD was strongly correlated to these compounds in muscle. In the second group, there were strong correlations between γ-HCH, 2,4'-DDE, cis-chlordane, CB-118 and BDE-28 in liver. In muscle, significant correlations were only found between γ-HCH and 2,4'-DDE, 4,4'-DDT and 2,4'-DDE, and between cis-chlordane and CB-118. The same correlations were highlighted using concentrations normalized with lipid content.

DISCUSSION

Few studies already focussed on Procellariiformes contamination in the southern Indian Ocean, including trace elements (Carravieri et al., 2013; Blévin et al., 2013; Bocher et al., 2003; Hindell et al., 1999) and POPs (Tanabe et al., 2004; Guruge et al., 2001a,b). However, to the best of our knowledge, this study is the first to investigate seabird contamination in the southern Indian Ocean by such a large number of contaminants and in such a large number of tissues.

4.1 Tissue distribution of trace elements: comparison with other seabirds and other areas

Essential element concentrations (As, Co, Cu, Fe, Mn, Se and Zn) in internal tissues and feathers were in accordance with most previous studies focusing on Procellariiformes from the Southern Ocean (e.g., Bocher et al., 2003; Lock et al., 1992; Jerez et al., 2013; Seco Pon et al., 2011). Surprisingly however, Cu and Fe concentrations in feathers were 30 and 100 times lower, respectively, than concentrations observed in Antarctic prions from South Georgia (Anderson et al., 2010). In this last study, values were characterised by standard deviations 3 times higher than the mean values highlighting a huge variation in their results. Moreover, feathers were cleaned, but not washed prior to analysis, which may have introduced some important errors into trace element results (Anderson et al., 2009). This bias could be particularly important for birds nesting in burrows or rock crevices, as Antarctic prions, which can be in direct contact with dust and rocks containing naturally these elements. In contrast with soft tissues and feathers, essential element concentrations in blood were not in accordance with most of the literature (e.g., González-Solís et al., 2002; Anderson et al., 2010), with higher concentrations than expected for Cu, Mn and Zn, and lower values for Fe. This could be linked to the fact that we collected blood from heart auricles of dead birds, instead of blood sampling from living animals. These birds probably died of exhaustion and may suffer from a period of starvation, and hence influencing on blood essential element concentrations. In this respect, previous research showed that body condition is one of the most important factors influencing essential element concentrations in blood, especially in the case of Cu, Fe and Zn (Debacker et al., 2000; Malinga et al., 2010). Results on essential element concentrations in blood should thus be interpreted with caution. Except for blood, no differences were found in essential element concentrations between Antarctic prions and seabirds feeding at higher trophic levels, such as albatrosses (e.g., Kim et al., 1998; Seco Pon et al., 2011). This is not surprising since essential elements are submitted to homeostatic

control. This process involves the regulation of their absorption, which in turn depends upon nutritional requirements of the individual (Walsh, 1990). As expected, Fe, Mn and Zn were preferentially accumulated in the liver. Thus, these essential elements appeared to be closely regulated in this tissue (Elliott and Scheuhammer, 1997). Higher concentration of Cu in muscle than in liver was however unexpected. This accumulation pattern has already been observed, although not explained, in Antarctic prions from Kerguelen Island (Bocher et al., 2003), and in Barau's Petrels (*Pterodroma baraui*) and Audubon's Shearwaters (*Puffinus lherminieri bailloni*) from the Réunion Island (Kojadinovic et al., 2007a). The Se distribution in soft tissues was in agreement with previous works showing that this essential metal is preferentially retained in kidney (e.g., Mendes et al., 2008; Kim et al., 1998). Tissue concentrations of non-essential elements Cd, Hg and Pb were within the ranges of concentrations reported elsewhere for other Procellariiformes, including Antarctic prions (Bocher et al., 2003; Kojadinovic et al., 2007a; Kim et al., 1998; Bond and Lavers, 2011; Anderson et al., 2009, 2010). However, Cd concentrations were higher in the Antarctic prion than those found in fish-eating seabirds (Agusa et al., 2005; Mendes et al., 2008). This is not surprising, since Cd is not biomagnified through food chains in marine environments, and marine animals at low trophic positions can show higher concentrations than those at high trophic positions (Sanchez-Hernandez, 2000). Instead, the high Cd burden of Antarctic prions could be explained by their important consumption of the amphipod *Themisto gaudichaudii* (Cherel et al. 2002), which shows high Cd concentrations in Kerguelen waters (Bocher et al. 2003). In the five tissues studied here, Hg concentrations were lower than those in fish- or squid-eating seabirds, such as albatrosses (Anderson et al., 2009). This is consistent with the Hg biomagnification within food webs, which leaves top predators at risk of high contamination levels through food intake (Furness and Camphuysen, 1997; Morel et al., 1998). Except in feathers, Pb concentrations were below the limit of quantification, as

previously shown (Jerez et al., 2013), likely because this non-essential element is preferentially accumulated in bone or feathers rather than soft tissues (Scheuhammer 1987). The preferential Cd accumulation in kidney, as shown here, is a usual trend in seabirds (Nam et al., 2005; Kojadinovic et al., 2007a; Mendes et al., 2008), and according to Scheuhammer (1987), a higher Cd concentration in kidney than in liver usually indicates chronic exposure to low Cd levels. As expected, the highest Hg concentration was found in feathers, since a high proportion of the Hg body burden can be excreted in the plumage during moult (up to 93%, Braune and Gaskin, 1987). Among soft tissues, liver presented the highest Hg concentrations, due to its important role in detoxification and storage of this non-essential element (Monteiro and Furness, 1995; Kim et al., 1998).

4.2 Tissue distribution and relative proportion of POPs: comparison with other seabirds and other areas

POP concentrations in Antarctic prions' tissues were low compared to most Procellariiformes breeding in the Southern Ocean. Because they biomagnify in food webs, POP concentrations are influenced by the diet and seabird trophic positions (Hop et al., 2002; Buckman et al., 2004; Borgå et al., 2005). Accordingly, ΣPCBs concentrations in Antarctic prions were similar to those of Antarctic petrels (*Thalassoica antarctica*) and Cape petrels (*Daption capense*) (van den Brink, 1997), which also feed mainly on crustaceans. On the other hand, ΣPCBs concentrations were lower than those of seabirds feeding at higher trophic levels, such as albatrosses and petrels from the Southern Ocean and Brazilian waters (Guruge et al., 2001a,b; Colabuono et al., 2012). Similarly, ΣOCPs concentrations in Antarctic prions were lower than those detected in albatrosses or skuas from the Southern Ocean (Guruge et al., 2001a; Corsolini et al., 2002), and similar to those in low trophic levels seabirds (Buckman et al., 2004; Mallory et al., 2004). Concentrations of OCPs and PCB congeners were generally

tended to be higher in liver (Table 2). This is consistent with the fact that levels of lipophilic pollutants, such as OCPs and PCBs, tend to be higher in organs with high metabolic activity (Malcolm et al., 2003). The highest contribution of CBs 138, 153 and 180 in Antarctic prions' tissues from Kerguelen Islands was similar to earlier data reported in various seabird species from the Southern Ocean (e.g., Court et al., 1997; Guruge et al., 2001a; Corsolini et al., 2011) and Arctic regions (e.g., Henriksen et al., 1998, 2000; Buckman et al., 2004). As described by Maervoet et al. (2004), the birds' capacity to metabolize PCBs decreases with increasing degree of PCB chlorination. Therefore, more chlorinated compounds like CBs 118, 138, 153 and 180 tend to be accumulated, while CBs 52 or 101 are more prone to metabolisation. The contribution of individual OCPs was also similar to earlier studies. 4,4'-DDE dominated the OCP pattern in Arctic and Antarctic seabirds, followed by HCB and Mirex (Borgå et al., 2001; Henriksen et al., 2000; Goerke et al., 2004). The particularly strong occurrence of 4,4'-DDE to OCPs in seabirds may be due to both the accumulation from the diet and from DDT metabolisation (Borgå et al., 2001). As shown by Sagerup et al. (2009), cis-chlordane, trans-nonachlor and γ-HCH presented lower liver concentrations than the most predominant OCPs, since these compounds seem to be more easily metabolised and excreted by seabirds (Borgå et al., 2001). As reviewed by Verreault et al. (2010), BDE-47 is largely the dominant PBDE in seabirds, followed by nearly equal contributions of BDEs 99, 100, 153 and 154. In this study, surprisingly high concentrations of BDE-209 were detected in the liver, muscle and kidney of three individuals. Law et al. (2006) reviewed that decabrominated biphenyls like BDE-209 are usually detected but always in low concentrations compared to BDEs 47, 99, 100 or 153. However, Lindberg et al. (2004) analysed PBDE in captive Peregrine falcon (*Falco peregrinus*) and highlighted a different congener profile for this population, dominated by BDEs 153 and 209. They explained this contrasting result by differences in exposure due to diet, although differences in metabolic capacity could be implicated. Moreover, all studies

reviewed by Law et al. (2006) focussed mainly on fish-eating birds while Antarctic prions feed mainly on zooplankton.Bureau (2001) showed that BDE-47 was the major congener found in three fish species (sprat, herring and salmon), while BDE-209 was present but in lower concentrations. On the other hand, BDE-47 and BDE-209 were in comparable concentrations in zooplankton. Therefore, the predominance of zooplankton in the diet of the Antarctic prion could partly explain its distinctive PBDE congeners' profile. However, high concentrations of BDE-209 were not exhibited in all individuals. Hence, these concentrations can also be due to individual variation in accumulation or detoxification mechanisms, and birds may have been exposed on local sources of PBDE in their wintering area (e.g., subtropical Indian Ocean, southern Australian coast or Tasmania; Marchant and Higgins, 1990) or along their migratory routes. Moreover, in this study, age and status of birds were unknown, and such individual traits may influence BDE-209 concentrations (Law et al., 2006).

4.3 Relationships among tissues and between contaminants

In the present study, a large number of positive correlations were observed between elements in Antarctic prions' tissues in accordance with previous studies in seabirds (e.g., Jerez et al., 2013; Mendes et al., 2008; Nam et al., 2005; Ribeiro et al., 2009). Concentrations of the trace elements Cu, Fe, Mn and Zn presented strong positive relationships (Cu-Fe, Cu-Mn, Cu-Zn, Fe-Mn, Fe-Zn and Mn-Zn), especially in livers and muscles. These correlations may suggest common sources of exposure, similar storage pathways and/or detoxification processes (Ribeiro et al., 2009). Additionally, these elements presented strong relationships with Cd. Previous studies have shown that Cd have similar regulation mechanisms to Cu and Zn, such as detoxification by binding to metallothioneins (MTs) and insolubilization in mineral concretions (Ikemoto et al., 2004; Kojadinovic et al., 2007b; Lucia et al., 2009, 2012). Many

studies have used blood and feathers to study trace element concentrations in seabirds in part because this non-invasive method allows a large sampling and therefore studies of bird populations (e.g., Burger and Gochfeld, 2004). Some authors have suggested that feathers are useful indicators of trace element concentration because the proportion of the body burden stored in the feathers is relatively constant for some elements, particularly Hg (Monteiro and Furness, 1995; Burger, 1993). In this study, Hg blood concentration, and As blood concentration in a lower proportion, appeared to be good indicators of soft tissue concentrations. Nonetheless, feather trace element concentrations, and particularly Hg, were not significantly correlated to the other tissues. Once the feather is formed, the feather no longer exchanges blood with soft tissues; the feather is effectively sealed off from the rest of the body physiologically, implying that no further element is deposited. In adult Antarctic prions, feather Hg concentrations had not evolved since the last moult, whereas Hg concentrations in the other tissues had increased with Hg bioaccumulation. Moreover, since in this flying seabird the feathers growth is asynchronous, feathers that grow at different times present different Hg concentrations, as body burden is progressively depleted during moult (Furness et al., 1986; Braune and Gaskin, 1987). In the case of species exhibiting low Hg concentrations, as Antarctic prions, concentrations in first moulted feathers indicate a combination of current dietary intake and Hg accumulated in the body between moults, while feathers grown at the end of moult indicate mainly current intake (Furness and Camphuysen, 1997). Hence, such within-individual variation hinders the use of feathers to predict Hg concentration in soft tissues. However, this problem can be resolved by using birds with synchronous or almost synchronous moult, like penguins or chicks (Carravieri et al., 2014; Stewart et al., 1999; Burger and Gochfeld, 2004). Similarly, once absorbed, Cd and Pb become firmly bound in kidney and bone, respectively, and only enter feathers in trace amounts (Walsh, 1990; Furness, 1993; Stewart et al., 1994). Therefore, as suggested by Nam

et al. (2005), given that there is a seasonal cycle of some element accumulations and elimination in the soft tissues relative to feather moult, it would be better to consider cautiously the use of feather element concentrations to predict concentrations in soft tissues. Concerning POPs, comparison of inter-tissue and inter-compound relationships with other birds is difficult given the scarcity of data. In this study, highly persistent PCBs (CBs 118, 138, 153 and 180) and OCPs (HCB, 4,4'-DDE and Mirex) presented strong inter-tissue correlation. High correlations of PCBs and DDTs between blood, fat, liver and muscle have been already reported in several seabirds including albatrosses and petrels (e.g., Henriksen et al., 1998; Colabuono et al., 2012). Similarly, these most persistent POPs where highly correlated in each Antarctic prions' tissue studied here (liver, kidney and muscle). Bustnes et al. (2001) and Mora et al. (1993) also highlighted strong correlations between CBs 118, 138, 153, 180, HCB and 4,4'-DDE in the blood of Glaucous gull (*Larus hyperboreus*) and Caspian tern (*Hydropogne caspia*). These compounds are highly persistent and concentrations among tissues are directly related when those contaminants reach the equilibrium between incorporation and excretion in the organism (Colabuono et al., 2012; Matthews and Dedrick, 1984).

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Table 1. Trace element concentrations (mean ± SD, µg g^{-1} dw; number of samples above the limit of detection (LoD) are given in brackets) in blood, liver, kidney, muscle and feathers of 10 Antarctic prions from Kerguelen Islands.

	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	V	Zn
Blood	<LoD	0.55 ± 0.20 (9)	1.20 ± 0.41 (10)	<LoD	0.10 (<LoD-0.10) (2)	2.46 ± 1.85 (10)	1620 ± 397 (10)	0.67 ± 0.11 (10)	0.65 ± 0.20 (10)	<LoD	<LoD	101.5 ± 33.3 (10)	<LoD	31.6 ± 10.2 (10)
Liver	<LoD	2.73 ± 0.82 (10)	35.6 ± 8.44 (10)	0.17 ± 0.05 (10)	2.67 ± 1.26 (10)	23.2 ± 5.56 (10)	2465 ± 863 (10)	1.82 ± 0.34 (10)	16.1 ± 3.92 (10)	1.06 ± 0.60 (10)	<LoD	72.9 ± 17.5 (10)	<LoD	249 ± 40.1 (10)
Kidney	<LoD	0.78 ± 0.25 (10)	105 ± 36.9 (10)	0.23 ± 0.04 (10)	3.05 ± 1.25 (10)	14.73 ± 2.91 (10)	568 ± 62.8 (10)	0.88 ± 0.16 (10)	6.87 ± 1.01 (10)	1.56 ± 0.70 (10)	<LoD	87.9 ± 16.8 (10)	<LoD	147 ± 18.1 (10)
Muscle	<LoD	0.93 ± 0.20 (10)	2.83 ± 0.81 (10)	0.10 ± 0.02 (10)	2.21 ± 1.06 (10)	37.2 ± 5.92 (10)	559 ± 108 (10)	0.28 ± 0.06 (10)	4.01 ± 0.64 (10)	0.83 ± 0.48 (9)	<LoD	33.66 ± 4.72 (10)	<LoD	91.9 ± 14.8 (10)
Feathers	<LoD	0.37 (<LoD-0.51) (2)	0.06 ± 0.03 (10)	<LoD	0.49 ± 1.05 (8)	6.05 ± 2.07 (10)	12.5 ± 5.44 (10)	2.83 ± 1.18 (10)	1.09 ± 0.68 (10)	0.71 (<LoD-1.34) (4)	0.15 ± 0.09 (10)	6.51 ± 2.52 (10)	<LoD	71.8 ± 19.1 (10)

Table 2. Persistent organic pollutant concentrations (mean ± SD, ng g^{-1} dw; number of samples above the limit of detection (LoD) are given in brackets) in liver, kidney and muscle of 10 Antarctic prions from Kerguelen Islands.

	CB-50+28	**CB-52**	**CB-101**	**CB-118**	**CB-153**	**CB-138**	**CB-180**	**Σ_7PCBs**
Liver	1.11 (<LoD-2.52) (5)	2.79 (<LoD-6.24) (5)	2.56 (<LoD-6.13) (5)	5.65 ± 2.03 (10)	14.04 ± 6.51 (10)	8.28 ± 3.51 (10)	2.54 ± 1.35 (10)	37.0 ± 16.33
Kidney	0.24 (<LoD-1.63) (1)	<LoQ	0.65 (<LoD-3.99) (1)	3.58 ± 2.41 (7)	10.21 ± 5.71 (10)	6.09 ± 3.36 (10)	1.73 ± 1.27 (10)	22.8 ± 11.99
Muscle	1.53 (<LoD-7.54) (6)	3.10 (<LoD-14.8) (5)	2.41 (<LoD-12.4) (5)	4.99 ± 2.75 (10)	11.89 ± 6.85 (10)	6.84 ± 3.82 (10)	2.18 ± 1.30 (10)	32.9 ± 18.2

	HCB	γ-HCH	Heptachlor	2,4'-DDE	Cis-chlordane	Trans-nonachlor	4,4'-DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT	Mirex	Σ_{12}OCPs
Liver	20.56 ± 7.87 (10)	0.58 ± 0.28 (9)	<LoD	0.96 ± 0.35 (10)	1.87 ± 0.88 (10)	1.03 ± 0.49 (10)	49.86 ± 50.5 (10)	<LoD	<LoQ	<LoQ	<LoQ	24.81 ± 16.57 (10)	102.7 ± 68.1
Kidney	15.86 ± 5.84 (10)	0.27 (<LoD-0.78) (5)	<LoD	0.43 (<LoD-1.07) (6)	1.02 ± 0.75 (7)	0.56 ± 0.40 (8)	32.39 ± 41.0 (10)	<LoD	0.46 (<LoD-2.87) (1)	<LoQ	<LoQ	22.77 ± 21.62 (10)	75.2 ± 60.07
Muscle	9.84 ± 2.27 (10)	0.53 ± 0.58 (9)	<LoQ	0.80 ± 0.93 (9)	1.27 ± 1.03 (10)	0.78 ± 0.28 (10)	45.9 ± 52.6 (10)	<LoD	2.24 ± 1.35 (8)	<LoQ	0.27 ± 0.22 (8)	21.55 ± 10.91 (10)	85.0 ± 63.5

	BDE-17	BDE-28	BDE-49+71	BDE-47	BDE-66	BDE-100	BDE-99	BDE-154	BDE-153	BDE-183	BDE-209	Σ_{12}PBDEs
Liver	<LoD	0.22 ± 0.14 (10)	<LoD	<LoQ	<LoD	<LoD	<LoD	0.04 (<LoD-0.26) (1)	0.31 (<LoD-1.50) (2)	0.34 (<LoD-2.33) (2)	115 (<LoD-1023) (3)	118 ± 326
Kidney	<LoD	<LoQ	<LoD	0.06 (<LoQ-0.13 (5)	<LoD	<LoD	<LoQ	0.33 (<LoD-0.18) (1)	0.13 (<LoD-0.75) (2)	0.20 (<LoD-1.37) (2)	35.5 (<LoD-321) (4)	36.5 ± 101
Muscle	<LoD	0.23 ± 0.37 (8)	<LoD	0.03 ± 0.02 (9)	<LoD	<LoD	0.02 (<LoD-0.04) (1)	0.06 (<LoD-0.30) (2)	0.27 (<LoQ-1.26) (4)	0.32 ± 0.62 (7)	4.13 (<LoD-25.6) (5)	5.80 ± 10.46

Fig. 1. Cd, Cu, Hg and Se concentrations (µg g^{-1}; dw: dry weight) in blood (B), liver (L), kidney (K), muscle (M) and feathers (F) of Antarctic prions from Kerguelen Islands (n=10).

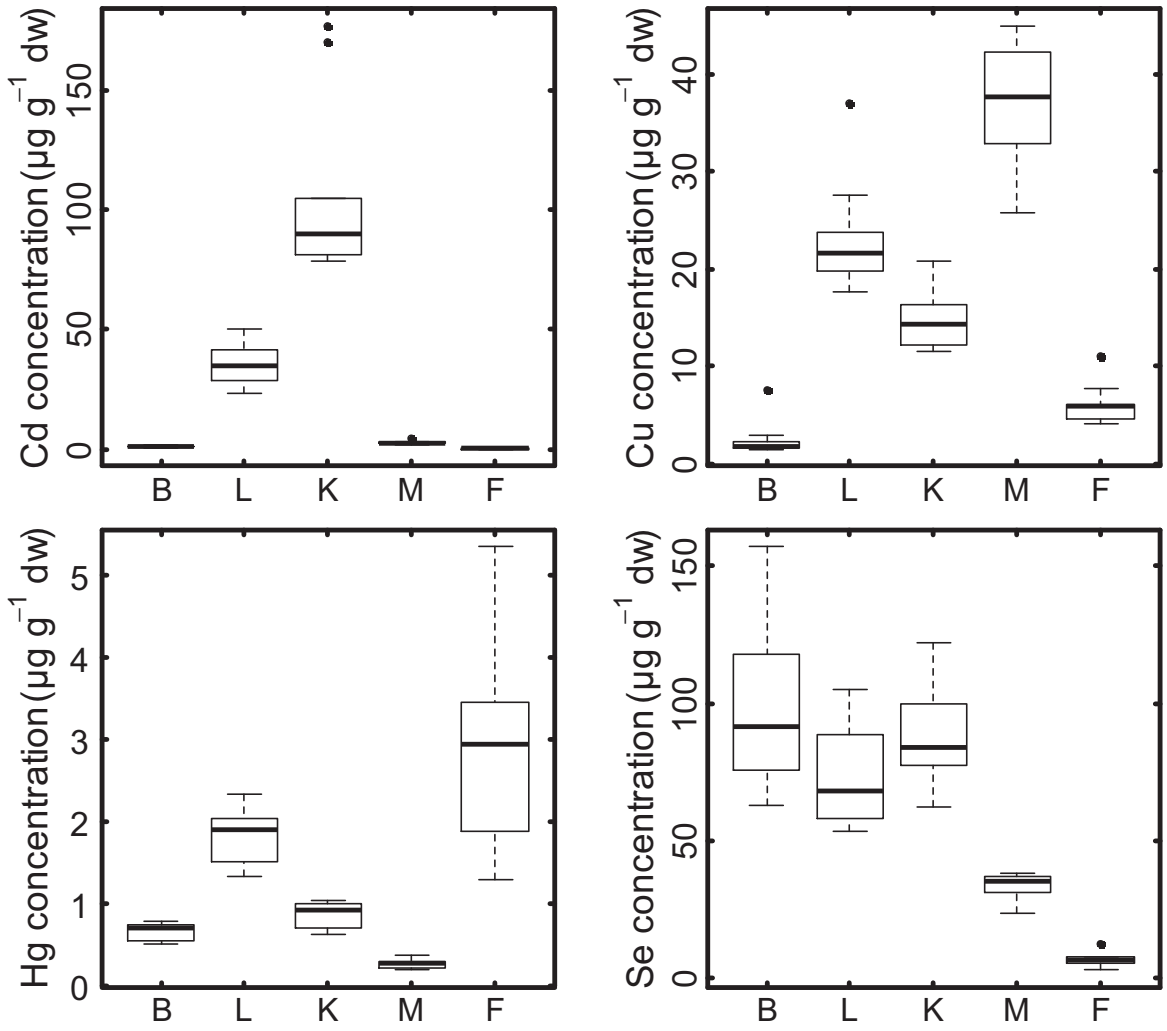


Fig. 2. Relationships between Hg concentrations (µg g^{-1}; dw: dry weight) of liver, kidney, muscle and feathers versus that of blood of Antarctic prions from Kerguelen Islands (n = 10).

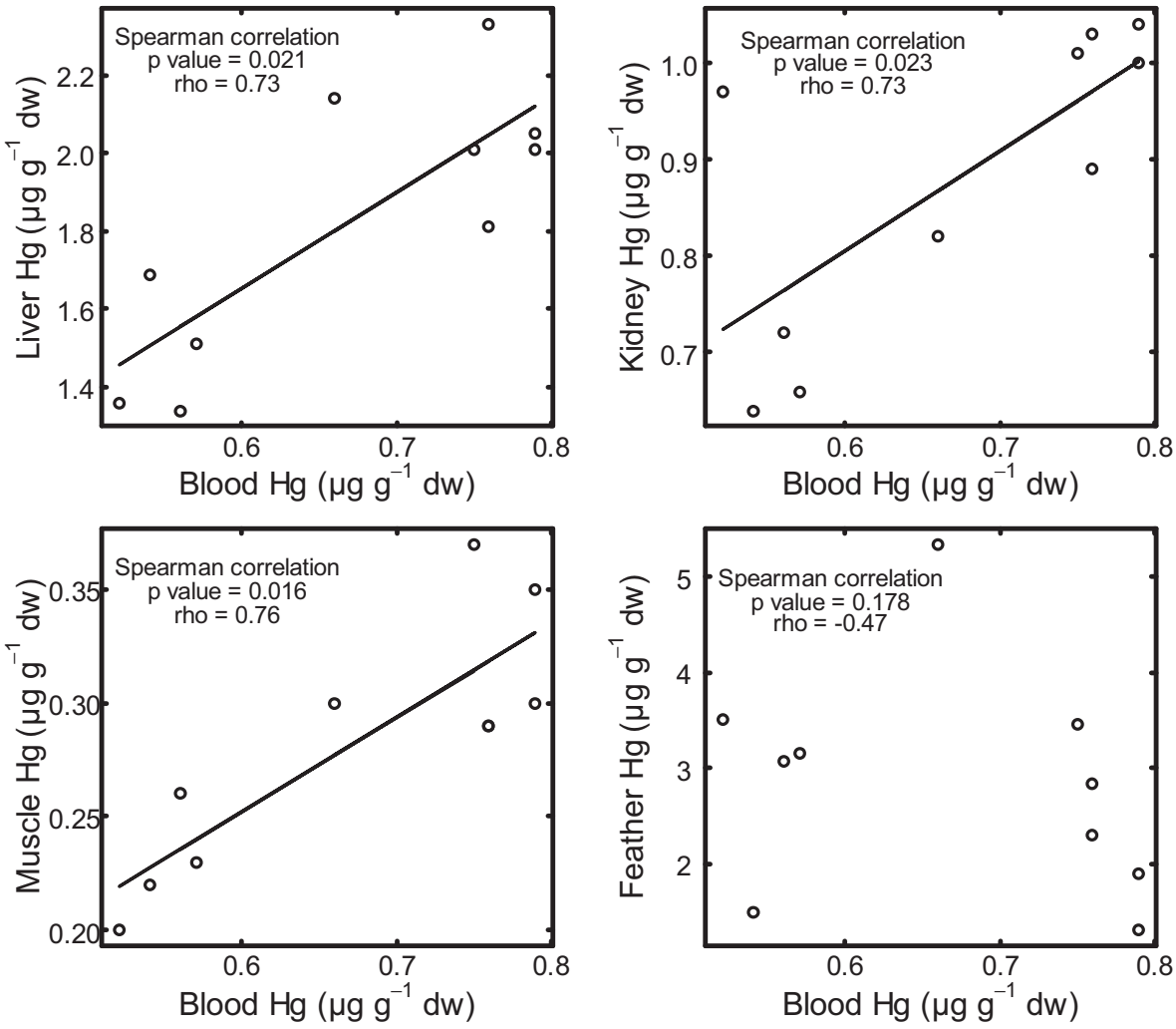


Fig. 3. $\sum_7$PCBs and selected OCPs (HCB, Mirex and 4,4'-DDE) concentrations (ng g^{-1}; dw: dry weight) in liver, kidney and muscle of Antarctic prions from Kerguelen Islands (n = 10).

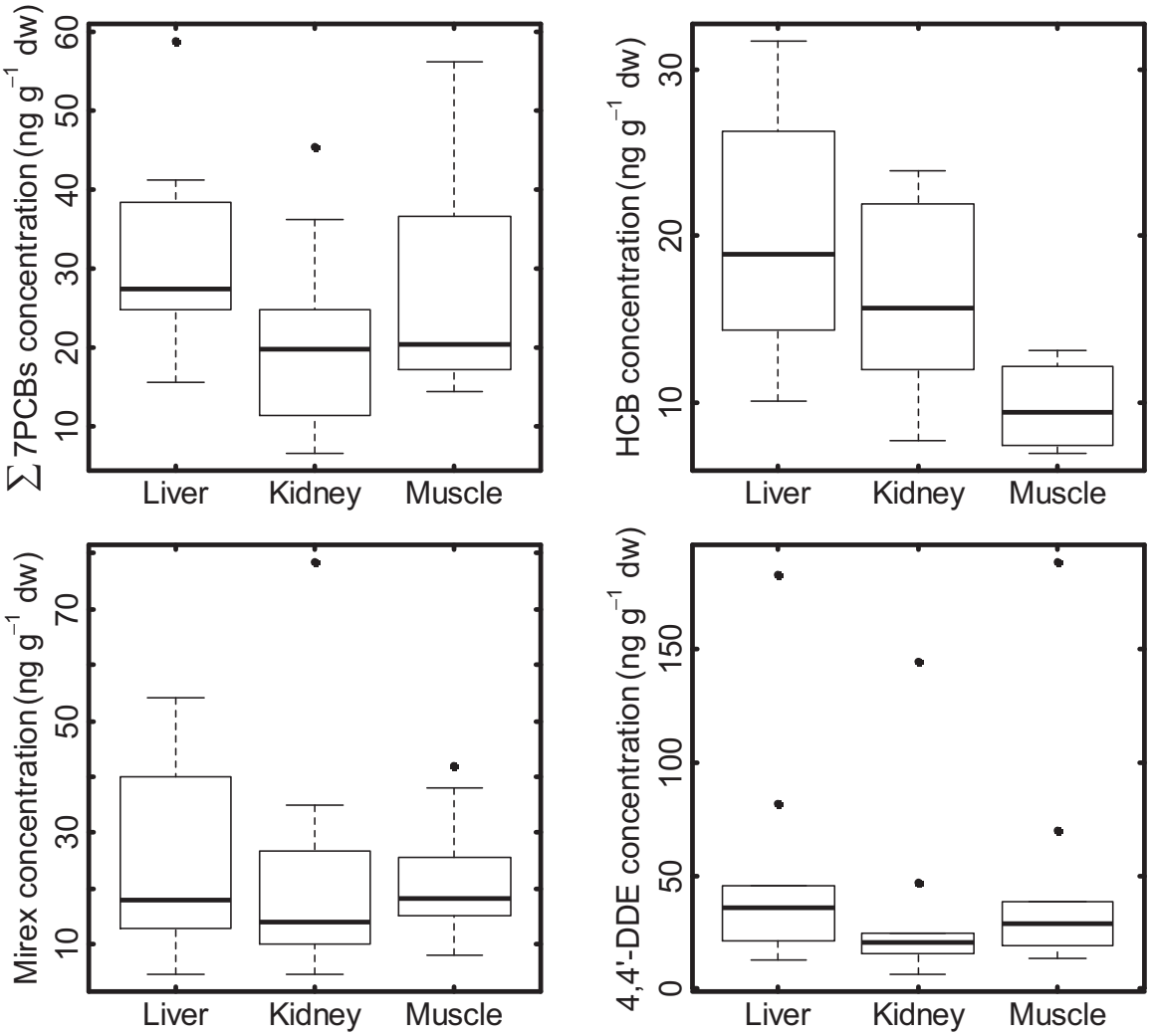


Fig. 4. Relationship between liver and kidney concentrations (ng g^{-1}; dw: dry weight) of $\sum_{12}$OCPs and CB-153 in Antarctic prions from Kerguelen Islands (n = 10).

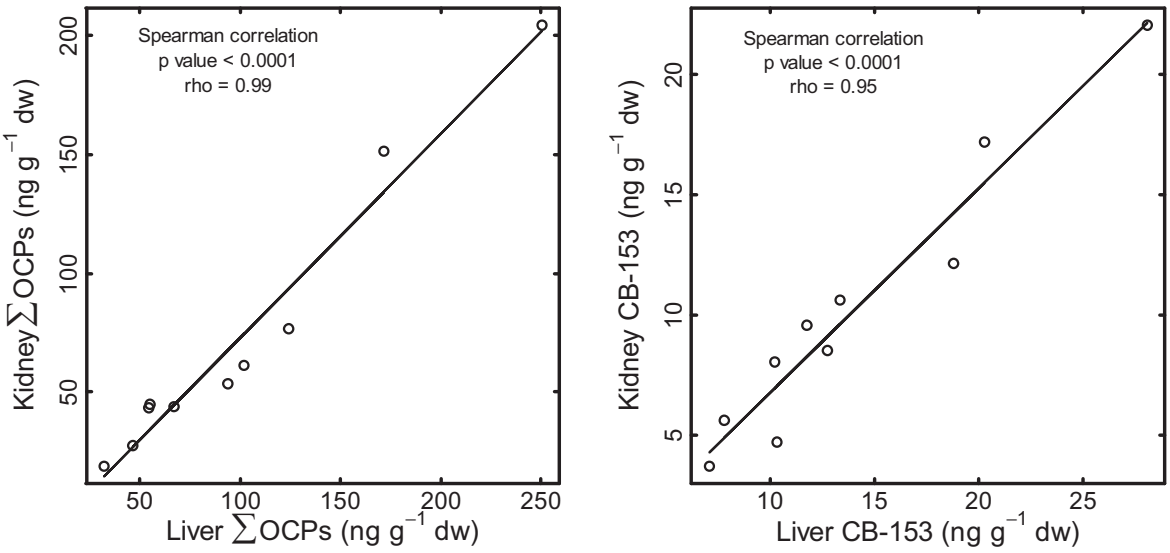
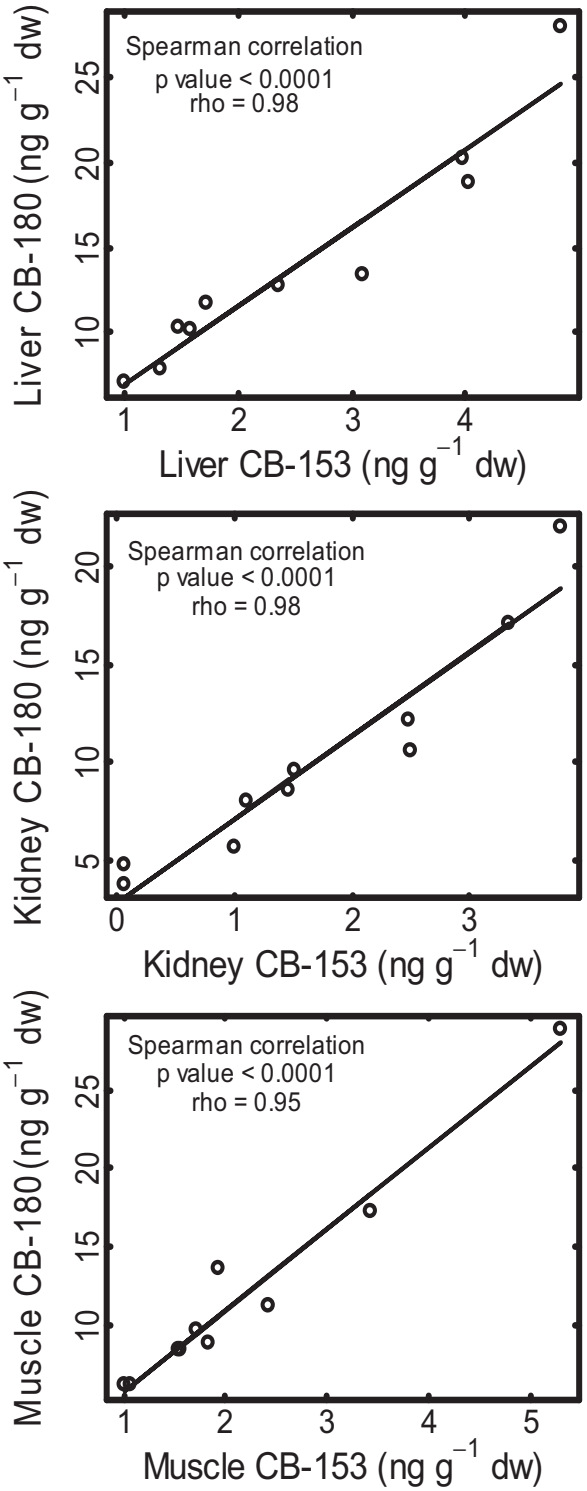


Fig. 5. Relationship between CB-153 and CB-180 concentrations (ng g^{-1}; dw: dry weight) in liver, kidney and muscle of Antarctic prions from Kerguelen Islands (n = 10).



Supplementary data

Table S1. Moisture and lipid content in tissues of Antarctic prions from Kerguelen Islands.

Tissues	n	Moisture (%)	Lipid content (%)
Blood	10	-	-
Liver	10	64.5 ± 1.3	13.1 ± 2.5
Kidney	10	69.3 ± 1.4	14.7 ± 2.4
Muscle	10	68.8 ± 1.1	9.6 ± 1.7
Feather	10	-	-

Table S2. Limits of detection (LoD) and limits of quantification (LoQ) of PCBs, OCPs and PBDEs for liver, kidney and muscle samples (pg g^{-1}, sample amount: 300 mg dw).

	CB-50+28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180					
LoD	10	30	25	20	8	10	8					
LoQ	350	100	800	700	270	300	270					
	HCB	**γ-HCH**	**Heptachlor**	**2,4'-DDE**	**Cis-chlordane**	**Trans-nonachlor**	**4,4'-DDE**	**2,4'-DDD**	**4,4'-DDD**	**2,4'-DDT**	**4,4'-DDT**	**Mirex**
LoD	15	30	130	100	40	15	200	350	250	200	280	280
LoQ	50	100	450	330	130	50	660	1200	850	670	470	950
	BDE-17	**BDE-28**	**BDE-49+71**	**BDE-47**	**BDE-66**	**BDE-100**	**BDE-99**	**BDE-154**	**BDE-153**	**BDE-183**	**BDE-209**	
LoD	30	30	30	30	30	20	20	30	20	20	200	
LoQ	90	90	90	90	90	60	60	100	70	70	700	

Table S3. Determination of POP levels in NIST SRM 1947 (trout muscle); *: certified concentration for CB-28 only; **: certified concentration for BDE-49 only.

Compounds	Certified concentration	Experimental concentration
CB-50+28	14.1 ± 1.0*	18.8 ± 0.5
CB-52	36.4 ± 4.3	23.9 ± 0.9
CB-101	90.8 ± 0.3	63.4 ± 1.4
CB-118	112.0 ± 6.0	77.0 ± 22.3
CB-153	201.0 ± 3.0	167.0 ± 5.6
CB-138	162.0 ± 6.9	172.2 ± 3.2
CB-180	80.8 ± 5.0	66.6 ± 2.0
HCB	7.48 ± 0.66	3.71 ± 0.46
trans-nonachlor	127.0 ± 6.0	137.7 ± 7.7
4,4'-DDE	720.0 ± 43.0	705.2 ± 9.7
2,4'-DDD	3.31 ± 0.16	0.92 ± 0.07
4,4'-DDD	45.9 ± 3.6	33.3 ± 0.9
2,4'-DDT	15.7 ± 0.89	20.8 ± 2.1
4,4'-DDT	59.5 ± 6.7	68.7 ± 4.5
Mirex	3.39 ± 0.28	2.49 ± 0.43
BDE-49+71	4.01 ± 0.10**	4.20 ± 0.26
BDE-47	73.3 ± 2.9	69.7 ± 8.3
BDE-66	1.85 ± 0.13	0.98 ± 0.16
BDE-100	17.1 ± 0.6	17.8 ± 1.6
BDE-99	19.2 ± 0.8	18.0 ± 2.6
BDE-154	6.88 ± 0.52	7.25 ± 0.68
BDE-153	3.83 ± 0.04	3.65 ± 0.47

Table S4. Limits of detection (LoD) of trace elements for blood, liver, kidney, muscle and feather samples (μg g^{-1} dw).

Elements	Blood	Liver	Kidney	Muscle	Feather
Ag	$0.3\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.2\ 10^{-1}$
As	$3.0\ 10^{-1}$	$3.8\ 10^{-1}$	$3.4\ 10^{-1}$	$3.4\ 10^{-1}$	$2.2\ 10^{-1}$
Cd	$0.3\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.2\ 10^{-1}$
Co	$0.3\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.2\ 10^{-1}$
Cr	$0.3\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.2\ 10^{-1}$
Cu	$1.5\ 10^{-1}$	$1.9\ 10^{-1}$	$1.7\ 10^{-1}$	$1.7\ 10^{-1}$	$1.1\ 10^{-1}$
Fe	5.9	7.5	6.9	6.8	4.3
Mn	$1.5\ 10^{-1}$	$1.9\ 10^{-1}$	$1.7\ 10^{-1}$	$1.7\ 10^{-1}$	$1.1\ 10^{-1}$
Ni	$0.6\ 10^{-1}$	$0.7\ 10^{-1}$	$0.7\ 10^{-1}$	$0.7\ 10^{-1}$	$0.4\ 10^{-1}$
Pb	$0.3\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.4\ 10^{-1}$	$0.2\ 10^{-1}$
Se	$1.5\ 10^{-1}$	$1.9\ 10^{-1}$	$1.7\ 10^{-1}$	$1.7\ 10^{-1}$	$1.1\ 10^{-1}$
V	$5.9\ 10^{-1}$	$7.5\ 10^{-1}$	$6.9\ 10^{-1}$	$6.8\ 10^{-1}$	$4.3\ 10^{-1}$
Zn	5.9	7.5	6.9	6.8	4.3
Zn	5.9	7.5	6.9	6.8	4.3

Abstract

Seabirds as bioindicators of Southern Ocean ecosystems: concentrations of inorganic and organic contaminants, ecological explanation and critical evaluation

Antarctic and subantarctic marine environments are reached by inorganic and organic contaminants through ocean circulation and atmospheric transport. Yet, environmental contamination is poorly known in the Southern Ocean, in particular in the Indian sector. Among environmental contaminants, **mercury (Hg)** and **persistent organic pollutants (POPs)** are primarily of concern, because they are toxic, highly mobile, and they bioaccumulate in the tissues of living organisms and biomagnify up the food web. **Seabirds**, as upper predators, are exposed to large quantities of contaminants *via* food intake and have widely served as biomonitors of marine contamination, notably through the non-destructive sampling of their **feathers** and **blood**. My doctoral work has focussed on the abundant and diverse seabird species (more than 40) breeding in the **French Southern and Antarctic Lands**, southern Indian Ocean, in order to describe and explain contaminant concentrations over a large latitudinal gradient, **from Antarctica to the subtropics**, and to identify the best bioindicator species for contaminant biomonitoring. In a first methodological step, seabirds with synchronous moult of body feathers (**adult penguins** and **chicks of all species**) were recognised as good candidates as bioindicators, because, unlike most adult birds, they present low within-individual variation in feather contaminant concentrations. In a second explanatory step, the influence of intrinsic (individual traits) and extrinsic factors (feeding ecology inferred from the **stable isotope** method) driving variation in contaminant concentrations was evaluated in feathers of the large avian community of the Kerguelen Islands (27 species) and in blood of wandering albatrosses from the Crozet Islands (180 birds of known individual traits). **Feeding ecology** was the main factor driving variation in contaminant concentrations of blood and feathers, both at the community, population and individual levels, whereas age, sex, phylogeny and breeding status played a minor role. Age-class was however an important intrinsic factor to consider, with chicks usually having lower concentrations than adults. In a third step, **spatio-temporal patterns** of contamination were studied through selected bioindicator species and by taking into account their feeding habits. Results from different species (oceanic seabirds) and populations (skua chicks) showed that, contrary to predictions, Hg transfer to seabirds gradually increases from Antarctic to subantarctic and subtropical waters, whereas, in accordance with the global distillation theory, POPs transfer has the opposite pattern. Comparisons between penguin feathers from museum collections and contemporary samples showed that Hg transfer to seabirds is overall not different today when compared to 50-70 years ago, but subantarctic species are possibly experiencing an increasing trend. Future research efforts should be focussed on the use of **feathers as biomonitoring tools**, in particular for POPs determination. The best recommended bioindicator species include the **emperor penguin** and **snow petrel** (Antarctic), **king penguin**, **blue petrel** and **black-browed albatross** (subantarctic), and **northern rockhopper penguin** and **Indian yellow-nosed albatross** (subtropical). Future biomonitoring studies on these species will give invaluable insights into the poorly-known temporal trends of environmental contamination in the Southern Ocean.

Key words: albatrosses, feathers, feeding habits, mercury, penguins, persistent organic pollutants, petrels, stable isotopes, trace elements.

Résumé

Les oiseaux marins, bioindicateurs des écosystèmes australs : niveaux de contaminants métalliques et organiques, explication écologique et évaluation critique

L'océan Austral est soumis à la redistribution globale des contaminants par les voies atmosphérique et océanique. Cependant, la contamination des écosystèmes australs est très peu connue, en particulier dans le secteur Indien. De par leur toxicité, leur mobilité et leur capacité à se bioaccumuler dans les tissus des organismes et à se bioamplifier dans les réseaux trophiques, le **mercure (Hg)** et les **polluants organiques persistants (POPs)** comptent parmi les contaminants les plus préoccupants. Du fait de leur position élevée dans les réseaux trophiques, les **oiseaux marins** sont exposés à de grandes quantités de contaminants par la voie alimentaire. En conséquence, ils sont souvent utilisés comme bioindicateurs de l'état de contamination des écosystèmes, par le biais des **plumes** et du **sang**, qui peuvent être échantillonnés de façon non destructive. Ma thèse s'est intéressée aux nombreuses espèces d'oiseaux marins (plus de 40) qui nichent au sein des **Terres Australes et Antarctiques Françaises**, au sud de l'Océan Indien, afin de décrire et expliquer les niveaux de contaminants le long d'un large gradient latitudinal, de l'Antarctique à la Zone Subtropicale, et d'identifier les meilleures espèces bioindicatrices pour un suivi à long terme de la contamination de ces écosystèmes. Au cours d'une première étape méthodologique, les **manchots** et les **poussins de toutes les espèces** ont été identifiés comme de bons bioindicators de contamination puisque, à la différence de la plupart des oiseaux adultes, ils présentent une faible variabilité des niveaux de contaminants dans les plumes. Au cours d'une seconde étape explicative, l'effet de facteurs intrinsèques (traits individuels) et extrinsèques (écologie alimentaire déduite grâce à la méthode des **isotopes stables**) sur les niveaux de contaminants a été évalué dans les plumes des oiseaux de la communauté de Kerguelen (27 espèces) et dans le sang du grand albatros de Crozet (180 individus dont les traits de vie sont connus). L'**écologie alimentaire** s'est avérée être le principal facteur explicatif des niveaux de contaminants, tandis que l'âge, le sexe, la phylogénie et le statut reproducteur jouent un rôle mineur. La classe d'âge est néanmoins un facteur à prendre en compte, puisque les poussins montrent souvent des concentrations inférieures aux adultes. Au cours d'une troisième étape, les **variations spatio-temporelles** de la contamination ont été étudiées en utilisant une sélection d'espèces bioindicatrices et en tenant compte de leur écologie alimentaire. Plusieurs résultats portant sur différentes espèces (oiseaux océaniques) et populations (poussins de skua) ont montré que, contrairement aux prédictions, l'exposition des oiseaux au Hg augmente graduellement des eaux Antarctiques aux eaux subantarctiques puis aux subtropicales, alors que l'exposition aux POPs, en accord avec la théorie de la distillation globale, montre la tendance inverse. D'autre part, la comparaison des concentrations en Hg dans les plumes de manchot, effectuée entre des spécimens de musée et des échantillons actuels, indique que leur exposition au Hg n'a pas changée depuis les années 1950-1970. Toutefois, des espèces subantarctiques montrent une tendance à la hausse. De futures études devraient viser à l'utilisation des **plumes comme tissu de référence** pour l'évaluation et le suivi de la contamination des écosystèmes, en particulier en ce qui concerne les POPs. Parmi les nombreuses espèces étudiées au cours de ces travaux de thèse, les bioindicateurs les plus pertinents se révèlent être le **manchot empereur** et le **pétrel des neiges** (Antarctique), le **manchot royal**, le **pétrel bleu** et l'**albatros à sourcil noirs** (subantarctique), le **gorfou sauteur subtropical** et l'**albatros à bec jaune** (subtropical). Le suivi à long terme de ces espèces permettra d'évaluer l'évolution temporelle de l'état de contamination de l'océan Austral.

Mots clé: albatros, écologie alimentaire, éléments trace métalliques, isotopes stables, mercure, manchots, pétrels, plumes, polluants organiques persistants.